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THE HISTOCHEMISTRY OF KERATINIZATION.

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KERATIN is the natural end-product of epidermal cells. It is not a single chemical compound, as keratins show great variation in composition. They are composed of long polypeptide chains held together by three types of cross linkage. The most characteristic is the disulphide bond, formed by the oxidation of two sulphhydryl groups belonging to two cysteine residues in adjacent polypeptide chains. The two sulphur atoms are joined with the elimination of two atoms of hydrogen, and thus one molecule of cystine is formed, having part of its molecule in one polypeptide chain and part in the other. Their high content of cystine sulphur distinguishes keratins from most other proteins. The other bonds are salt linkages and hydrogen bonds. The former are ionic attractions between one carboxyl group of a dicarboxylic amino-acid contained in one polypeptide chain and an amino group of a diamino amino-acid in the opposite chain. A hydrogen bond is a link between two dipoles, one a hydrogen atom, the other usually an atom of oxygen or nitrogen.

The chemical changes that occur in the protoplasm of an epidermal cell during its transformation to keratin are not well understood. The main alterations appear to take place at the granular layer. We have therefore studied this layer by fluorescence microscopy and employed specific enzymes and histological methods for the localisation of enzymes and other substances. We have compared our findings with those in abnormal parakeratotic keratin. In addition, the effects of pathological and experimental pressure have been investigated by the same techniques.

NORMAL EPIDERMAL KERATINIZATION.

ENZYME STUDIES.

Fluorescence of the Granular Layer.

Titan yellow causes the granules to fluoresce golden yellow. This is characteristic, as no other material in skin fluoresces this colour. (Appendix ; Fluorochrome Technique No. 1). The effects of ribonuclease, deoxyribonuclease and lipase on this fluorescence have been investigated.

Ribonuclease (RNase). This enzyme was employed in a 0.04% aqueous solution at pH 6.5 with phosphate buffer. Alcohol fixed sections were taken to water, incubated at 37° C. for 1 hour and then fluorochromed with titan yellow. No alteration of the granular fluorescence resulted.

Deoxyribonuclease (DNase). Sections were digested for 1 hour at 37° C. in a 0.002% enzyme solution buffered to pH 6.5. This failed to alter the yellow fluorescence of the keratohyalin granules, but the blue nuclear fluorescence was considerably reduced.

Lipase. Sections were incubated for 15 minutes at 37° C. in a 0.04% aqueous solution of lipase (L. Light and Co. Ltd.) buffered to pH 8.0. Control sections were incubated in distilled water buffered to the same pH. This caused the complete disappearance of the golden fluorescence of the keratohyalin granules. It is therefore possible that these granules are composed of a complex lipid. They cannot be simple lipids, as the sections had already been treated with several fat solvents including alcohol, benzene, and xylol.

It seemed possible that the lipase was contaminated with proteolytic enzymes and that this test was therefore not conclusive evidence of the nature of the granules. Incubation with 0.002% trypsin, however, failed to abolish the fluorescence of the granules and this concentration would correspond to 5% contamination of the lipase with proteolytic enzymes. Higher concentrations of trypsin caused separation and destruction of the epidermis.

The findings with these three enzymes are similar to those reported using Harris's haematoxylin for the demonstration of the granules (Jarrett and Hardy, 1957). We have found no evidence that they are composed of ribonucleic acid, as has been suggested by Leuchtenberger and Lund (1951).

Distribution of Phospholipids.

The nature of the keratohyalin granules was further investigated by Baker's acid haematin method for phospholipids (Baker, 1944). A band of blue coloration results in the region of the granular layer and another on the outer surface of the keratin (Fig. 1). This outer band may be due to contamination of the surface with sebum. Red blood cells, eosinophils and medullated nerves also stain blue, owing to their content of phospholipid.

The absence of phospholipids higher in the keratin is probably due to their destruction during keratinization. This has already been indirectly demon-

strated by Snider *et al.* (1949) by estimating liberated choline. They considered that the latter was derived from the breakdown of phospholipids. It is known that phospholipids take part in oxidative and reductive cellular processes and it is thought that these compounds may supply energy for the formation of cross-linkages and the polymerization of the polypeptide chains during the formation of the keratin molecule. We modified Baker's technique for use with fixed and processed tissue and think that this demonstrates phospholipoprotein. (Appendix.)

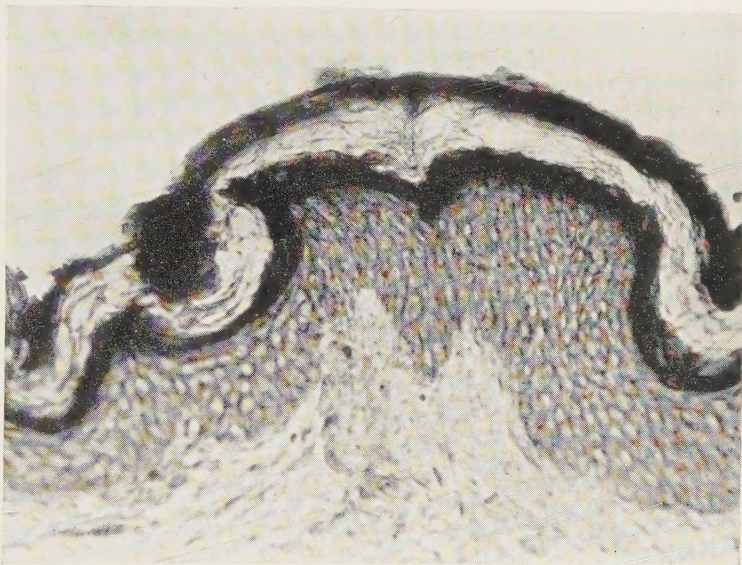


FIG. 1.

Dopa Studies. (Dihydroxyphenylalanine.)

The presence of oxidative reactions in the region of the granular layer was investigated by means of a modified dopa technique.

Routine alcohol fixed, paraffin embedded sections were taken to water and then incubated for 6 hours in a 0.2% aqueous solution of dopa buffered with phosphate at pH 7.4. Control sections were incubated in water buffered to the same pH. Melanocytes were not turned black by this procedure: the dopa oxidases usually present in these cells might have been destroyed when the tissue was impregnated with wax at 60° C. or during processing. Nevertheless, the granules in the keratohyalin layer became black as did the red blood cells and eosinophils. The darkening at these sites was considered to be due to oxidases persisting in the three sites that are already known to contain phospholipids. Because the dopa oxidases in the melanocytes had probably been inactivated by processing the tissue, we thought that the oxidisers remaining in the granular layer were either more resistant than those in the melanocytes, or not enzymatic.

Detection of Alkaline Phosphatase Activity.

The presence of alkaline phosphatase in normal skin was investigated by the cobalt technique and the silver modification (Lillie, 1952). The latter method was more satisfactory for the investigation of keratinization as it did not give false positives with inner root sheath keratin. The positive results obtained in this region may be due to the affinity of cobalt for this keratin and may be related to the high phosphate content of the inner root sheath. The silver technique failed to show the presence of alkaline phosphatases in the granular layer of human skin. In the tail skin of the rat, however, the granular cells in the vicinity of the follicular openings gave a positive reaction.

Alkaline phosphatases are inhibited by cysteine (Gomori, 1952). As this compound occurs in high concentration in the region of the granular layer, it was thought worth while to block the active sulphydryl groups with iodoacetic acid before examining for the presence of alkaline phosphatases. Paraffin sections of normal human skin were taken to water and immersed in a 0.2 M solution of iodoacetic acid that had been titrated to pH 8 with 0.2 N sodium hydroxide. The sections were left in this solution for one week, after which they were examined for the presence of alkaline phosphatases by the silver technique. A faint but definite positive reaction was found in the granular layer.

The demonstration of alkaline phosphatases after blocking sulphydryl groups may explain the positive reaction obtained in the follicular region of rat's tail skin, where the staining for sulphydryl groups is very much less intense than in the intervening keratin.

Detection of Acid Phosphatase Activity.

The presence of acid phosphatase activity in the granular region of human skin was clearly shown by the lead method of Gomori (1941). The α -naphthol phosphoric acid technique (Pearse, 1953) also gave positive results: but the method did not appear to be so reliable as that of Gomori and there was much greater diffusion of the coupled staining reaction.

The Effects of Acid and Alkline Phosphatases on the Phospholipid Content of Normal Skin.

Because it was thought that acid phosphatase in the granular layer of human skin might be responsible for the breakdown of phospholipids during keratinization, its effect on these compounds in skin was investigated.

Acid phosphatase was used in a concentration of 0.08% buffered with veronal acetate to pH 5.6. Fresh tissue was incubated in this solution for two hours at 37° C. The tissue was fixed in 70% alcohol and then stained for phospholipids by the modified Baker technique. This failed to show any alteration of phospholipid content. The experiment was repeated in the presence of magnesium ions in order to activate acid

phosphatase, but again without success. A further series of experiments was performed, incubating unfixed sections in the enzyme followed by staining by Baker's original technique. This failed to alter the content of phospholipids as compared with control sections or those treated with fluoride inhibitor. Fixed and processed sections were subjected to enzymatic digestion for varying periods of up to sixteen hours, but these also gave negative results. This series of experiments was repeated with alkaline phosphatase at pH 7.5 and this enzyme also failed to remove the material responsible for the positive reaction for phospholipids.

Combination of Trypsin and Acid Phosphatase.

In view of the negative results, we thought it possible that phospholipids were present in the form of phospholipo-proteins from which the enzyme could not remove the phosphate radicle. Attempts were then made to disrupt the molecule by predigestion with weak trypsin (0.008%) prior to the use of acid phosphatase, but this also failed to remove the phospholipids.

Combination of Lipase and Acid Phosphatase.

Fixed and processed sections were incubated at 37° C. for three minutes in 0.01% lipase solution buffered to pH 8. Control sections were incubated in water buffered to the same pH. Some of the sections treated with lipase were rinsed in water at pH 5.6 and transferred to acid phosphatase solution in a concentration of 0.08% at pH 5.6. These were incubated at 37° C. for two hours. The sections were stained for phospholipids by the modified Baker technique.

In the control sections phospholipids were still present whereas they were absent from those treated either with the lipase or lipase and acid phosphatase. We were therefore unable to demonstrate that phosphatases are capable of acting on the phospholipids in the granular layer. Although they are removed by lipase we have been unable to detect this enzyme in the granular layer by the method described by Gomori (1945).

Sulphydryl and Disulphide Groups.

The technique of Barnett and Seligman (1952) employing 2,2' dihydroxy 6,6' dinaphthyl disulphide was used for the demonstration of protein bound sulphydryl groups. This technique gave much better results than any other method we had previously tried. In normal human epidermis, keratin stained weakly for these groups but the region of the granular layer stained deeply.

The decrease in protein bound sulphydryl groups above the granular layer supports the conception that these groups are oxidized to disulphide linkages.

Disulphide groups were detected by a modification of the method described by Barnett and Seligman (1954). In this technique, sulphydryl groups are blocked by iodoacetic acid, and disulphide groups are then reduced with ammonium sulphide to sulphydryls which are detected by the technique mentioned above. Ammonium

sulphide was used in preference to other reducing agents because it caused less damage ; that it can form sulphydryl groups from active carboxyl groups was not thought to be a serious disadvantage as we were only interested in keratin.

A very high content of disulphide groups was demonstrated in hair keratin. Epidermal keratin also was positive, but stained much less intensely.

FLUORESCENCE MICROSCOPY OF NORMAL HUMAN KERATINS.

Alcohol fixed and processed normal human skin was studied by a series of fluorochrome techniques. The fluorochromes found to be of value in the study of keratins were rhodamine B (Appendix ; Fluorochrome Technique No. 2), I.C.I.8 (*do.*, No. 3), 9-phenyl-5,6-benzo-iso-alloxazine (*do.*, No. 3). Thioflavine T (*do.*, No. 5) caused yellow fluorescence with inner root sheath keratins and blue with epidermal and hair keratins : the significance of these findings has already been reported (Jarrett, 1958).

An extremely valuable fluorochrome method for the study of keratins was the combination of congo red and thioflavine T (Appendix ; Fluorochrome Technique No. 4). This combination causes different fluorescent colours with the various keratins. Thus, normal epidermal keratin is red or reddish-purple, inner root sheath keratin is yellow and hair keratin blue. Abnormal keratins can also be detected by this method and these will be discussed in more detail in the section on parakeratotic keratins.

I.C.I.8 and phenyl-iso-alloxazine both fluoresce with keratin. This appears to be the only structure in skin that fluoresces with these fluorochromes : however, not all keratins do so. Some parakeratotic keratins fail to fluoresce, but this does not seem to be related to the high —SH content of this type of keratin. We have been unable to demonstrate what linkage is responsible for the fluorescence, but it appears likely that it is related to the nature of the keratin. The more stable a keratin, the more strongly it fluoresces. Further work on these fluorochromes is being undertaken.

NORMAL EPIDERMAL KERATIN OF THE RAT AND MOUSE.

Skin from 30 albino house mice and 7 Wistar Norway rats was examined. The skin from the back of these animals was found to have a continuous granular layer similar to that of human epidermis. The keratin of this region is also histochemically similar to that of human skin. The tail skin of both animals, however, is quite different from normal human skin. In the regions of the hair follicles the epidermis has a well marked granular layer : in the intervening portions no granular layer is present. The keratin overlying the latter portions often stains with a different intensity with eosin from that around the follicles. The keratin formed in the interfollicular area is known as the "tail scale"

and is intermediate in type between normal human epidermal keratin and parakeratotic keratin. It gives the same blue fluorescence with congo red and thioflavine T as parakeratotic keratin and has the same high content of phospholipids and protein bound sulphydryl groups. There are, however, no remains of nuclear chromatin in the keratin. The perifollicular keratin which forms with a granular layer fluoresces the same colour as normal human keratin, and has a similar content of phospholipid and protein bound sulphydryls (Fig. 7).

The difference in cystine content of the two keratins in rodents' tail skin was examined by peracetic acid oxidation followed by fluorochroming with thioflavine T. (Appendix Fluorochrome Technique No. 6). The method was modified from the methods of Fraser *et al.* (1954) and Jarrett (1958). Normal keratin does not fluoresce yellow with thioflavine T, but does so after treatment with peracetic acid, owing to the oxidation of cystine. The intensity of this yellow fluorescence may be related to the initial cystine content of the keratin. It was observed that after treatment with peracetic acid, tail scale keratin fluoresced a more brilliant yellow than perifollicular keratin. This suggests that the tail scales have a higher content of cystine than other keratin formed with a granular layer. It would therefore appear from our investigations of these keratins that tail scale keratin has a higher content of both protein bound sulphydryl and disulphide groups than the keratin surrounding the follicles. This is of interest as it shows that there is not necessarily a reciprocal relationship between these two groups in keratin: this will be discussed in more detail later.

Tail scale keratin can readily be transformed into parakeratotic keratin by simple mechanical stimulation and we have used this property for the investigation of parakeratosis.

ABNORMAL KERATINIZATION.

Parakeratosis in Psoriasis.

In this disorder there is abnormal keratinization as is shown by a change in the physical state of the horny layer, the absence of a granular layer and the presence of nuclear remains in the epidermal keratin. The chemical and physical differences of psoriatic and normal keratin have been investigated by previous workers (Van Scott and Flesch, 1954; Magnus, 1956) who found an increased amount of free sulphydryl groups in the former. This was thought by Flesch to indicate an incomplete type of keratinization. Kooyman (1932) and Snider *et al.* (1949) both found that phospholipids decreased during the process of normal keratinization, but in the pathological accelerated keratinization of psoriasis this breakdown of phospholipids was incomplete (Snider *et al.*, 1949).

By means of histochemical techniques we confirmed the biochemical findings of Van Scott and Flesch, Magnus, and Snider *et al.* The sulphhydryl content of psoriatic keratin was studied by the method described by Barnett and Seligman (1952). The keratin over active psoriatic areas stains more intensely

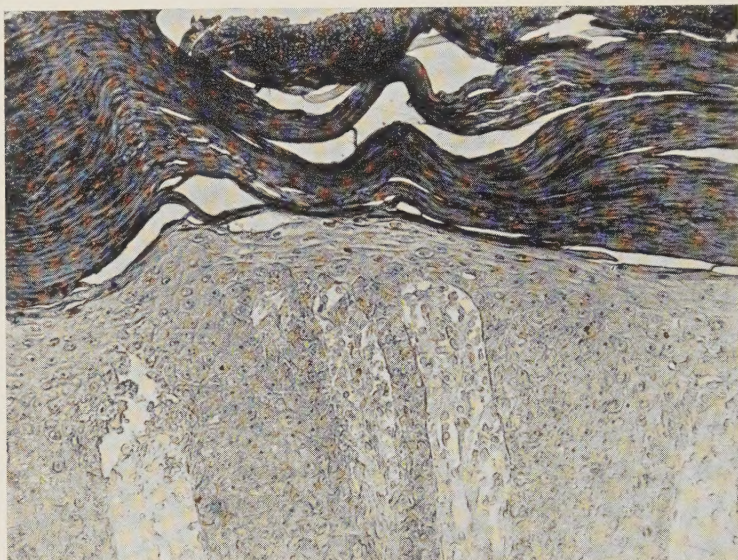


FIG. 2.

for sulphhydryl groups than that over normal epidermis. Psoriatic keratin also contains an increased amount of phospholipids as shown by a diffuse and intense staining reaction with Baker's acid haematin method for phospholipids (Fig. 2). This finding indicates that phospholipids have been incompletely

EXPLANATION OF PLATE.

- FIG. 3.—Normal human skin. Congo red, thioflavine T. The epidermal keratin fluoresces red-mauve, the protoplasm of the epidermal cells is red and the nuclei yellow. The collagen immediately beneath the epidermis is whitish-blue.
- FIG. 4.—Pinched human skin. Congo red, thioflavine T. The epidermal cell protoplasm now fluoresces blue and the collagen immediately below the epidermis is bright blue. The nuclei are compressed and fluoresce yellow.
- FIG. 5.—Psoriatic human skin. Congo red, thioflavine T. The bright blue fluorescence of the psoriatic keratin is clearly seen; the nuclear remains fluoresce yellow.
- FIG. 6.—Intraepidermal carcinoma. Congo red, thioflavine T. The keratin on the right of the picture fluoresces blue, and as the carcinoma spreads this overlying abnormal keratin gradually replaces the normal keratin on the left.
- FIG. 7.—Normal epidermis of mouse's tail. Congo red, thioflavine T. The keratin in the region of the hair follicles fluoresces red similar to normal human keratin. The intervening keratin of the tail scales is blue and thus resembles parakeratotic keratin except that there are no nuclear remains. In the dermis a hair follicle can be seen; the keratin of the inner root sheath fluoresces yellow.
- FIG. 8.—Base of a common wart. Rhodamine B. Keratin and nuclei fluoresce salmon pink with this fluorochrome. The cells in the lowest part of the wart adjacent to the dermis are becoming keratinized and are strongly fluorescent.

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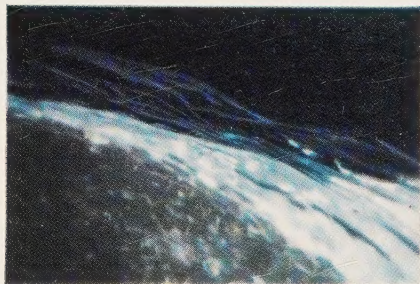
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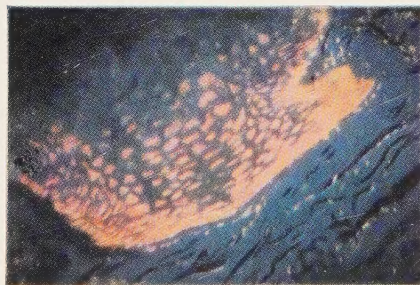
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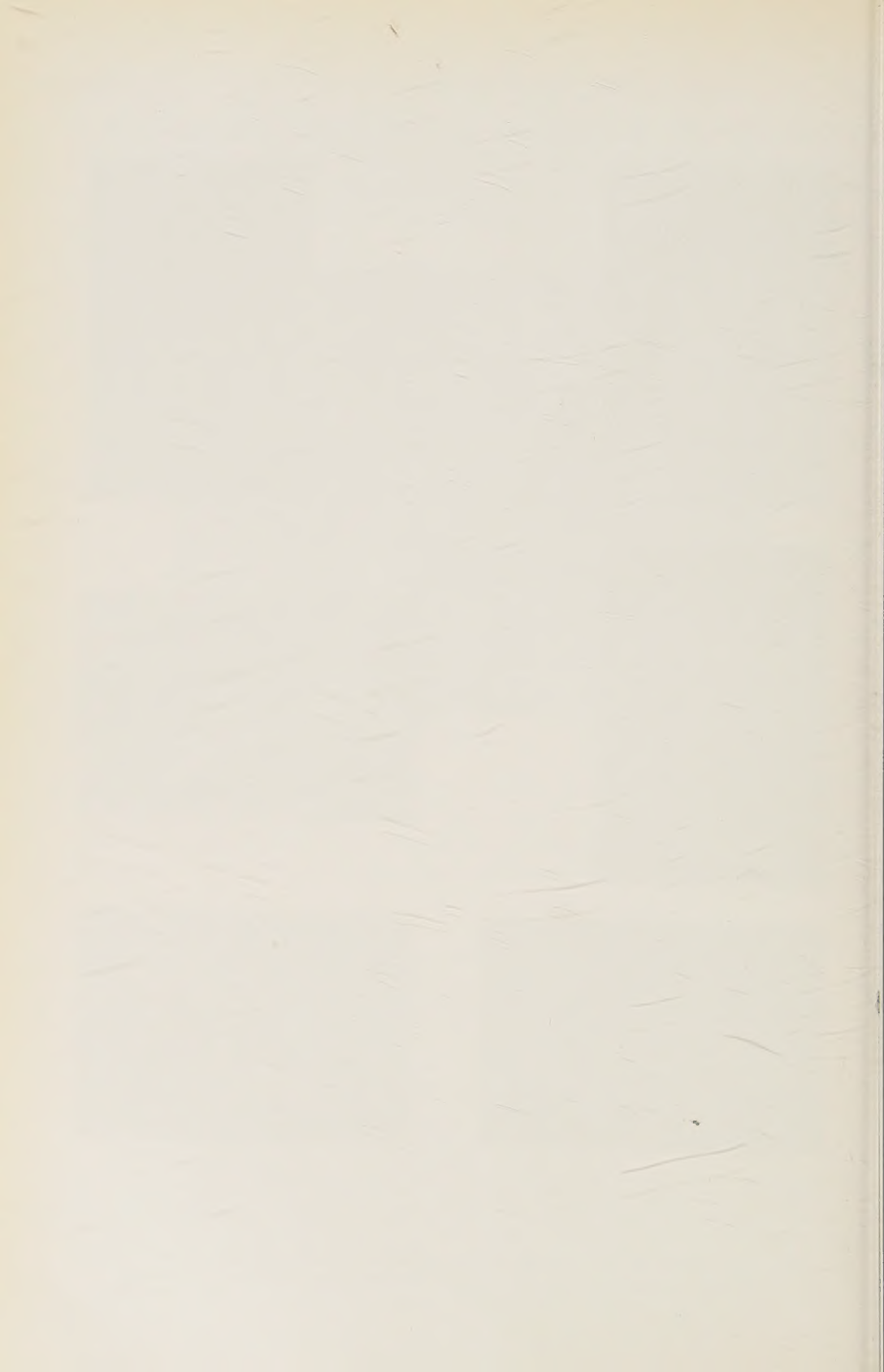


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removed during the formation of this abnormal keratin. The high content of sulphhydryl groups and phospholipids confirm the findings reported by Hollander *et al.* (1954). The keratin fluoresces blue with the congo red and thioflavine T technique and the nuclear remnants in the keratin fluoresce yellow (Fig. 4). This parakeratotic keratin can readily be distinguished from normal epidermal keratin. As no granular layer was present, the dopa technique previously described failed to show the presence of oxidases and there was no area in the epidermis positive for acid phosphatases. It is therefore possible that because of the absence of oxidases and enzyme activity in the epidermis, the phospholipids are not utilized and are carried up into the keratin layer. They are probably chemically bound to the keratin molecule as they are not removed by the usual lipid solvents and are detectable by our modification of Baker's technique. Owing to incomplete breakdown of phospholipids there is less energy available for the polymerization of the polypeptide chains of the keratin molecule and this would result in a keratin with abnormal physical properties.

Abnormal Keratinization in Warts.

The keratin of warts was studied by the same methods as those used for psoriatic keratin. Warts have an abnormal granular layer: it is increased in depth and the "keratohyalin" granules are abnormal. They have been mistaken for inclusion bodies. At the apices of some of the upward projecting epidermal cones the granular layer is absent, and above these points there are columns of parakeratotic keratin. This has the same histochemical features as psoriatic keratin: the intervening keratin varies in its phospholipid content and in its intensity of staining for sulphhydryl groups. The granular layer contains dopa oxidases and acid phosphatases, but the reactions for these appear to be rather less intense than in normal epidermis. There is also evidence of keratinization of the deeper epidermal cells penetrating the dermis: this type of keratinization will be described more fully later.

Abnormal Keratinization over Malignant Epidermis.

Over areas of rapidly proliferating epidermis, as in squamous-cell and intra-epidermal carcinomas, there is often abnormal keratinization. It is usually of the parakeratotic type and has the same histochemical features as psoriatic keratin (Fig. 6). Occasionally a peculiar type of keratinization occurs in frankly malignant epitheliomas where there is complete keratinization of individual cells, so that the keratin layer consists of a mosaic of keratinized pieces. That these are not composed of normal keratin is shown by the results of special fluorochroming methods. The congo red and thioflavine T blocking technique gives the same colour of fluorescence as that of parakeratotic keratin, but there are no fluorescent nuclear remains.

PRESSURE KERATINIZATION.

Epidermal Keratinization in Benign Epidermal Hyperplasia.

During the investigation of benign epidermal hyperplasia by fluorescence microscopy it was observed that the deeper layers of epidermal cells penetrating the dermis fluoresced like keratin. This occurred with several fluorochrome techniques and was most frequently seen in warts (Fig. 8). This basal keratinization was also present in some cases of psoriasis, squamous papilloma, molluscum contagiosum and keratoacanthoma. In fact, any benign acanthoma may exhibit it, but we have never observed it in frankly malignant invasions of the dermis. Such areas of keratinization stain strongly for phospholipids. They also fluoresce yellow with thioflavine T, indicating the presence of nucleic acids. This keratin therefore resembles that of inner root sheath. Both are formed by pressure and without a granular layer: this will be discussed more fully in the section on experimental animal keratinization. Only minimal changes in the basal region of these hyperplasias are seen in haematoxylin and eosin sections as a slight increase in cytoplasmic eosinophilia and some compression of the nuclei.

This keratinization may be due to pressure exerted by the deeper dermis upon the downward growing epidermal cells. In normal skin, the collagen deep in the dermis is different in structure from that immediately beneath the epidermis. It appears that the thrust of the proliferating epidermal cells into this deeper collagen results in their becoming keratinized. Malignant epidermal growths that deeply invade the dermis are ensheathed in the superficial type of collagen and this does not appear to initiate keratinization of epidermal cells. The effect of this pressure on benign epidermal hyperplasia is to halt the downward growth and probably this is one of the mechanisms that prevents dermal invasion by the epidermis.

EXPERIMENTAL PRESSURE.

The Effect of Brief Pressure on Living Human Epidermis.

Brief pressure was applied to living human skin by means of forceps. The pinched area and an adjacent piece of normal skin were then removed surgically and fixed in 70% alcohol. Paraffin sections were prepared, and examined by routine and fluorochrome techniques. The congo red, thioflavine T method showed gross changes in the pinched area. In normal skin before pinching the cytoplasm of the epidermal cells fluoresces red, nuclei are yellow and the dermis blue (Fig. 3). After pinching, the cytoplasm was bright blue and some of the yellow nuclear fluorescence had diffused into cytoplasm (Fig. 4). In addition, the degree of orientation of the collagen as seen by polaroscopy was greatly increased. Slight changes could be detected with haematoxylin and eosin. These were an increase in the basophilia of the epidermal cells and flattening of the nuclei. These were insufficient to attract attention had not obvious changes been seen by fluorescence microscopy. The pinched area was positive for phospholipids by Baker's acid haematin method. This was not a false positive due

to liberated nuclear proteins, as it remained after digestion with both RNase and DNase.

These findings show that changes can readily be detected by fluorescence microscopy after very brief pressure on epidermis. The alterations must have occurred very rapidly, as the time from pinching the skin to its being placed in fixative was less than 3 minutes. The significance of these findings and their relation to more prolonged experimental pressure on animal epidermis will be discussed later.

Prolonged Experimental Pressure on Animal Epidermis.

The differences in chemical composition between the keratins of inner root sheath and epidermis have already been reported (Jarrett, 1958). These are thought to be due to different modes of keratinization. Inner root sheath keratin is formed without the intervention of a granular layer and Auber (1952) suggested that this type of keratinization was initiated by pressure exerted within the turgid follicle cells. We therefore thought that pressure on epidermal cells, if sufficiently prolonged, might cause them to become keratinized. The type of keratin produced under these conditions should resemble inner root sheath keratin rather than normal epidermal keratin. It would produce a keratin that still retained the nuclear protein of the cells. This is the type of keratin formed in benign epidermal invasion of the dermis and in this respect it is also similar to inner root sheath keratin.

We investigated this hypothesis by clamping rubber bungs split lengthwise on to rats' tails in order to exert a constant pressure on the skin. The sides of the tail were compressed by the two halves of the bung held together by fine wire. By this means a small area of skin on either side of the tail was subjected to pressure, leaving the dorsal and ventral surfaces unconstricted. This allowed some blood flow to the tail to continue and avoided producing ischaemic effects on the tissues as far as possible. Two animals were killed after 24 hours, four after 48 hours, and six after 72 hours. Skin was taken from the pressure sites and from control sites proximal and distal to the compressed areas. After 48 hours' pressure, cells in the basal layer showed changes similar to those seen in the base of a wart. Sections stained with haematoxylin and eosin showed increased eosinophilia of the epidermal cells in the pressure sites; these same cells also retained the violet staining with Gram's method of normal epidermal keratin. The pressure areas fluoresced with L.C.I.S. phenyl-iso-alloxazine, thioflavine T, and rhodamine B. After 72 hours, the changes extended upwards from the basal layer to the stratum corneum, so that the whole of the epidermis had become keratinized. The altered prickles were flattened with shrunken nuclei and the cytoplasm had an increased nucleic acid content as judged by the thioflavine T and RNase technique. After 48 hours' pressure, the birefringence of the epidermal cells was reorientated. Usually the molecular orientation under polarised light is at right angles to the keratin layer. As a result of pressure, the birefringence became much more marked and was parallel to the surface.

These findings show that the type of keratin in the inner root sheath and at the base of benign epidermal hyperplasia can be produced experimentally by

pressure on normal epidermal cells. The formation of this keratin does not require the intervention of a granular layer.

The effects of prolonged experimental intradermal pressure.

Pressure was applied to growing hair follicle cells by means of deep dermal injections of low melting point paraffin wax. The injections were made at body temperature into the backs of three rats and three mice. Twelve days before injection, hair growth on the whole back was stimulated by manually plucking the hairs (Montagna, 1956). The paraffin wax was injected into one side of each animal's back; the other side served as a control. A hump was raised and by this means the pressure in the dermis was increased. After twelve days the animals were killed and the skin was removed from the injected and control sides.

The effect was a great increase in the amount of inner root sheath keratin of the hair follicles and they became out of phase with the growing follicles on the control sides. There were also proliferative changes in the epidermal cells and these are being investigated further.

DISCUSSION OF RESULTS.

Epidermal Keratinization.

There is evidence of a high energy system in the region of the granular layer of normal human skin, and that this is responsible for the synthesis of the keratin molecules from the cytoplasmic polypeptides of the epidermal cells. The oxidation of cysteine to the disulphide amino-acid cystine is an exothermic reaction and Ellis *et al.* (1950) suggested that instead of this conversion requiring external energy it was actually capable of making a contribution to the system. Cysteine can be converted *in vitro* into cystine by magnesium. The reaction occurs spontaneously with the liberation of heat and the formation of hydrogen peroxide (Neurath and Bailey, 1954). It appears therefore that the energy liberated at this site is not required for simple oxidation, but is probably used in the synthesis and polymerization of polypeptide chains and for the removal of chemically combined water.

Sulphydryl groups, phospholipids and glycogen are concentrated in the granular layer and they are all absent in the immediately overlying keratin. The intense staining reaction for sulphydryl groups is probably due to the unfolding of the polypeptide chains of the cytoplasm, thus increasing the number of reacting sulphydryl groups (Magnus, 1956). Higher up in the epidermis these unfolded chains are broken down and resynthesized into the keratin molecule. This would account for the reduction in the intensity of the staining for sulphydryl groups: an additional reason for this reduction might be the presence of the enzyme cysteine desulphydrase. This forms

pyruvate from cysteine, the sulphur being removed as inorganic sulphates. The pyruvate can then be built up into glycogen and in this way cysteine can be converted into glycogen (Baldwin, 1957).

Phospholipids are probably formed at the time of nuclear breakdown. If the nucleotide, cytidine triphosphate, were liberated from the nuclei, it could be used in the formation of phospholipids. This compound combines with phosphoryl choline to form a diphosphate complex which in turn reacts with a diglyceride to form lecithin and cytidine monophosphate. Phospholipids can be utilized as a source of phosphate in the reformation of the high energy phosphate bonds in the adenosine triphosphate enzyme system. It has been shown by Snider *et al.* (1948) that there is a breakdown of phospholipids during keratinization and we have been able to show by histochemical methods that these compounds disappear immediately above the granular layer. The presence of glycogen in this site (Rothman, 1954a) indicates that this compound may be used as a source of energy.

When we compare normal human epidermal keratin with parakeratotic or rodent tail keratin, both of which are formed without a granular layer, we find that there are interesting histochemical differences. In parakeratotic and rat tail keratin there are disulphide bonds and as previously stated it appears that external energy is not required for their formation. These also have a higher concentration of sulphydryl groups than normal epidermal keratin, which may be due to the incomplete breakdown and resynthesis of the unfolded polypeptide chains of the epidermal cytoplasm. The high content of phospholipids in parakeratotic keratin is evidence of incomplete utilization of the high energy system: this results in the formation of a keratin that is not highly polymerized and is therefore physically different from normal epidermal keratin. Its water content is probably also greater (Rothman, 1954b) and these factors might account for the lack of adhesion of parakeratotic scales.

It appears that keratinization is not simply the formation of bonds between preformed polypeptide chains already present in epidermal cell protoplasm. It is more likely that at first there is an active breakdown and resynthesis of these chains which are then highly polymerized. All these alterations apparently take place in the region of the granular layer. Interference with this mechanism at any level results in the production of an abnormal keratin. Thus abnormalities of epidermal proteins, abnormal rates of epidermal growth, or enzymatic deficiencies may all cause the production of altered keratin. Parakeratotic keratin seems to result from a greatly increased rate of epidermal cell proliferation and because of this there is insufficient time for nuclear breakdown, or for the breakdown and resynthesis of the polypeptides. Epidermal proliferation is probably more important in the production of psoriatic parakeratosis than any specific enzyme defect. This type of keratinization is commonly seen over other proliferating epidermal conditions such as carcinomas,

warts and senile keratoses and in all the parakeratotic keratins are histochemically similar.

Another point of interest revealed by the study of parakeratosis is the independence of the sulphydryl and disulphide contents of keratin. Although sulphydryl groups are used in the formation of disulphide bonds they do not necessarily bear a reciprocal relationship to each other. In parakeratotic keratin it is quite possible to have a high sulphydryl content together with a high disulphide content. The high sulphydryl content is due to the presence of unfolded polypeptide chains that have not been resynthesized into the more highly polymerized molecule of normal keratin.

The physical durability and chemical stability of keratins depend on two main factors, the linkages between the polypeptide chains and the degree of polymerization of the chains. These two factors may be dissociated and a durable keratin can be produced by a high content of disulphide bonds, as in human hair. Conversely, normal epidermal keratin has fewer disulphide linkages, but its stability and pliability is probably due to a high degree of polymerization.

Pressure Keratinization.

Inner root sheath keratin has a different chemical constitution from normal epidermal keratin: it is thought to be formed by pressure on the epidermal cells of hair follicles. It is accordingly produced without the intervention of a granular layer and although the nuclei are morphologically destroyed, the nuclear proteins both of the cytoplasm and nuclei are not broken down completely but are incorporated into the keratin molecule (Jarrett, 1958).

This type of keratin appears to be very similar both in its formation and chemical constitution to that formed by pressure on the lower parts of a benign epidermis invading the dermis: both have nucleic acids and a high phospholipid content. It appears from these observations that pressure alone is sufficient to initiate keratinization of epidermal cells. This keratin contains most of the original contents of the cell. We know that phospholipids are rapidly formed as a result of pressure. Their formation appears to follow the disruption of the nuclei and these complex lipids are perhaps formed by way of the nucleotide cytidine triphosphate, which is liberated into the cytoplasm as a result of damage to the nucleus. It is possible that pressure causes the death of the cells, followed by the formation of disulphide linkages across the polypeptide chains. This does not require an enzyme system and can occur without external energy. Other cell constituents are incorporated into the molecule: thus the nuclear proteins become an integral part of the keratin molecule and this nucleic acid content is one of its main distinguishing features from normal epidermal keratin.

This concept of pressure keratinization has been verified by animal experiments. It was shown that external pressure on rat's tail skin caused the deeper epidermal cells to keratinize. As a result of prolonged pressure, the whole thickness of the epidermis became keratinized and this was found to be similar to inner root sheath keratin in that it contained nucleic acids and had similar fluorescence characteristics. We have also demonstrated that deep pressure applied by dermal injection of low melting point wax caused a great increase in the amount of inner root sheath keratin in hair follicles. These findings confirm the view that epidermal cells can undergo keratinization from pressure alone and that this is the probable mechanism of the formation of inner root sheath keratin. This type of keratinization is also probably the limiting factor that prevents dermal invasion by a proliferating epidermis that has not become frankly malignant.

TABLE I.—*Comparison of Different Keratins.*

	Human st. corneum rat and mouse back.	Rat and mouse tail scales.	Human para- keratotic keratin.	Inner root sheath keratin.	Hair (excluding medulla)
Haematoxylin and eosin	pink	pink	pink	pink	light pink
Nuclear chromatin haematoxylin and eosin	absent	absent	++	absent (nuclei in rodents)	absent
Congo red, thioflavine T	red or mauve	blue	bright blue	yellow	blue
I.C.I.8	green	green	green or nil	green	green
—SH groups	+	++	+++	++	neg.
Phospholipids	neg.	++	++	++	neg.
Ribonucleic acid	neg.	neg.	+	++	neg.

SUMMARY.

Normal and pathological human epidermis has been subjected to a series of histochemical, fluorochrome and enzyme processes, in an attempt to study some of the aspects of keratinization.

The main changes occur at the granular layer. The formation of the disulphide and other linkages appears to involve exothermic reactions and therefore does not require external energy. These reactions occur readily in the presence of trace metals. There appears to be breakdown of the pre-existing polypeptide chains in the epidermal cytoplasm which are then resynthesized into the keratin molecule. It is for this resynthesis and subsequent polymerization that external energy is required and there is evidence of a high energy system located in the region of the granular layer.

Different keratins have different modes of formation. Inner root sheath keratin appears to be formed by pressure and has a different chemical structure from either hair or normal epidermal keratin. As it is formed by pressure, it has similar properties to that formed at the base of non-malignant hyperplastic epidermis invading the dermis. It is possible that this basal keratinization is one of the methods whereby the dermal spread of benign growths is prevented.

Parakeratotic keratin has also been examined by these methods and the differences between this and normal epidermal keratin are described.

Experimental epidermal keratinization has been studied in rats and mice. External pressure applied to the epidermal cells results in their becoming keratinized. The type of keratin produced is similar to that of inner root sheath. We have also produced keratinization of follicular epidermal cells by increasing intradermal pressure by the injection of low melting point paraffin wax.

The mechanisms of keratinization are discussed in the light of these findings.

A summary of the histological and histochemical differences of the various keratins is given in Table I.

We are most grateful to Dr. W. N. Goldsmith for his helpful criticism. We also thank Mr. A. Bligh for his help with the photomicrographs and the coloured photofluoromicrographs. R. I. Spearman receives a personal grant from the Medical Research Council.

APPENDIX.

FLUOROCHROMES.

It is necessary to fix skin in 70% absolute alcohol for fluorochrome techniques as formalin is unsatisfactory. The method of processing has been reported previously (Jarrett *et al.*, 1956). Sections were cut at 7μ , dewaxed in xylol and taken to distilled water prior to fluorochroming.

Fluorochrome technique No. 1.—Titan Yellow.

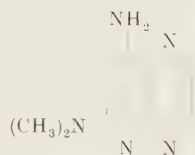
This fluorochrome induces a brilliant golden fluorescence of the granular layer and this is the only structure in skin to fluoresce in this manner. Sections taken from distilled water were treated with a 0.1% aqueous solution of the fluorochrome for 10 minutes. They were then rinsed in distilled water, rapidly dehydrated in 98% and absolute alcohol, cleared in xylol and finally mounted in the non-fluorescent mountant "DePeX". After enzyme incubation the sections were rinsed in distilled water and then fluorochromed as above.

Fluorochrome technique No. 2.—Rhodamine B.

This fluorochrome causes the nuclei and epidermal and inner root sheath keratin to fluoresce bright salmon pink. The cytoplasm of the epidermal cells, hair keratin and collagen are a dull greenish blue. This fluorochrome can be used to detect the early keratinization resulting from pressure on epidermal cells. Sections were brought to water and fluorochromed in a 0.1% aqueous solution for 3 minutes. They were then rapidly dehydrated, cleared and mounted as in the previous technique.

Fluorochrome technique No. 3.—I.C.I.8.

This compound was kindly supplied by Dr. F. L. Rose of I.C.I. Dyestuffs Division; its chemical formula is given below.



This compound causes bright green fluorescence with certain keratins. In skin, keratins are the only structures to fluoresce. Some keratins, however do not, for example parakeratotic keratins. We had not been able to detect the chemical grouping in keratin that determines whether fluorescence occurs: it does not appear to depend on the presence of a high content of disulphide groups, nor is it inhibited by compounds containing many free sulphhydryl groups.

Sections brought to 70% alcohol were fluorochromed for 6 minutes in a saturated 70% alcoholic solution. They were then rapidly dehydrated and mounted as in the other methods.

9-phenyl-5,6-benzo-iso-alloxazine (kindly supplied by Professor A. Haddow) causes keratin to fluoresce in a very similar manner and this can be used as an alternative to I.C.I.8.

Fluorochrome technique No. 4.—Congo Red, Titan Yellow, Thioflavine T Blocking Method.

By fluorescence microscopy it has been observed that congo red is taken up preferentially by certain tissues in the skin. Tissues thus stained are blocked in the sense that the entrance of subsequently applied fluorochromes is prevented. In the first attempts we employed congo red in an aqueous dilution of 1 in 10,000 for 25 minutes, followed by thioflavine T (0.1%) for 3 minutes. This method proved unstable and constant results were difficult to obtain. It appeared that a certain degree of maturation of the congo red was necessary in order to produce satisfactory reproducible results. This maturation was so unreliable that other methods of stabilisation were investigated. It was found after prolonged trials that the addition of 10 ml. of 0.1% titan yellow to 20 ml. of 1 in 20,000 aqueous solution of congo red produced stable and repeatable results. We found however that it was still advisable to make up the diluted congo red from a 1% stock solution and allow the diluted stain to mature for at least 24 hours before use. The titan yellow was added immediately prior to immersing the sections in the mixed fluorochromes.

Alcohol fixed paraffin sections were taken to water and then immersed in this fluorochrome mixture for 40 minutes. They were then rinsed in distilled water and fluorochromed for 3 minutes in a 0.1% aqueous solution of thioflavine T. After this they were rapidly rinsed, dehydrated, cleared and mounted as in the previous techniques. With this method the granular layer fluoresces brick-red, normal epidermal keratin red or red-mauve, inner root sheath keratin brilliant yellow and hair keratin light blue. Thus these keratins may easily be distinguished. Nucleic acids fluoresce brilliant yellow and this is due to the thioflavine T, which has a specific affinity for these compounds. In active epidermal cells the nucleoli are readily seen and the epidermal cytoplasm is greyish-blue. In normal resting epidermis there are very few nucleoli; the nuclei fluoresce yellow and the cytoplasm red.

Fluorochrome technique No. 5.—Thioflavine T.

This fluorochrome has been previously described in connection with the investigation of inner root sheath keratin (Jarrett, 1958). It appears to fluoresce specifically with nucleic acids. It is unable to distinguish between ribonucleic and deoxyribonucleic acids, but the distinction can readily be achieved by the use of specific enzymes. Thus after RNase digestion one can demonstrate DNA and after digestion with DNase the remaining RNA can be detected. This method is probably one of the most reliable means of demonstrating nucleic acids. The fluorochrome was used in a 0.1% aqueous solution and sections were immersed in this for 3 minutes, rinsed rapidly, dehydrated, cleared and mounted as in the other techniques.

Normal epidermal and hair keratins fluoresce blue, but inner root sheath keratin is brilliant yellow. The nuclei in parakeratotic keratin are easily seen because of their nucleic acid content. This method also detects the early pressure keratinization which like inner root sheath keratin fluoresces yellow.

Fluorochrome technique No. 6.—Peracetic acid oxidation for cystine.

Paraffin sections were taken to water and then immersed in 3% peracetic acid for 30 minutes. Sections were well washed in distilled water and fluorochromed with 0.1% aqueous solution of thioflavine T for 3 minutes. Keratins containing cystine fluoresce yellow; this may be due to oxidation products of cystine. Regions of high cystine content fluoresce a brighter yellow after oxidation than those of low cystine content.

ACID HAEMATIN STAIN FOR PHOSPHOLIPIDS.

Baker's original method was used initially for the detection of phospholipids in human and animal skin (Baker, 1944). The distribution of these compounds in human skin has already been described. As this method requires special formalin fixation which renders the tissue unsuitable for investigation with fluorochromes, we modified the original method for use with alcohol fixed tissue. The use of lipid solvents during the processing of tissues naturally reduced the phospholipid content; but a remarkable amount remained and its distribution was the same as that seen in tissue treated by Baker's original method. We therefore decided to use this modified technique for the detection of phospholipids in tissues that were being investigated by fluorescence microscopy.

This modified technique is capable of demonstrating differences in the phospholipid content of various keratins. Thus the alternation of positive staining of the tail scales and negative staining of the perifollicular keratin is readily seen in rat's tail skin. Parakeratotic keratin shows much greater intensity of staining than normal epidermal keratin.

Method (modified for alcohol fixed paraffin sections).

Sections are brought to water. They are immersed in a solution of potassium dichromate and calcium chloride for three hours at 60° C. and then overnight at 37° C. They are then washed in tap water for 10 minutes, rinsed in distilled water and placed in Baker's acid haematin for 3 hours. After this they are rinsed and immersed in a solution of borax and potassium ferricyanide for $\frac{1}{2}$ to $2\frac{1}{2}$ hours at 37° C. Finally, the sections are washed for 10 minutes in several changes of tap water and mounted in glycerine jelly from distilled water. The solutions were used in the concentrations recommended in Baker's original method.

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THE HUMAN TOE NAIL. ITS GENESIS AND BLOOD SUPPLY.

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THE NAIL is formed from an invagination of epithelium near the distal end of the terminal phalanx. Traditionally, the nail plate is held to arise from a matrix which consists of that portion of the invagination which lies between its apex proximally and the anterior margin of the lunula distally. According to Pinkus (1927), a small portion of the roof of this invagination may also on occasion form part of the matrix. The remainder of the floor of the invagination distal to the lunula and the greater part of the roof do not contribute to the nail plate. The eponychium arises from the epidermis of the roof and extends for a short distance on to the nail plate. The lateral nail folds contribute a small amount of eponychium (Pillsbury *et al.*, 1956).

Lewis (1953 and 1954) has challenged the traditional view, maintaining that the nail consists of three parts, dorsal, intermediate and ventral. He distinguishes these three by the results of silver proteinate staining. Furthermore, he states that in the intermediate nail the method of keratinization as determined by individual cell morphology and arrangement differs from that of the dorsal and ventral nail. The dorsal nail arises from the posterior half of the roof of the nail fold, the intermediate from the region traditionally known as the matrix and the ventral from the distal half to two-thirds of the nail bed immediately below the visible nail. Both the dorsal and the ventral nail may be partially or totally absent.

The genesis of the human nail plate is, therefore, disputed. The traditional theory and the considerations of Lewis are not diametrically opposed but are not in complete accord. The purposes of this study were to re-investigate the genesis of the nail plate, using additional techniques in order to demonstrate histochemically differences in the process of keratinisation within the various parts of the nail fold and to investigate the vascular supply of the nail plate. It was thought that the morphology of the vascular supply might shed additional light on the genesis of the nail plate.

MATERIAL AND METHODS.

Specimens consisted of toes removed from 13 amputated limbs, the digits of 2 full-term foetuses and several foetuses aged 3 to 5 months. Eleven of the adult

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limbs were removed on account of arteriosclerotic disease. The other two limbs were removed because of neoplasms in patients aged 45 and 48 years respectively. In these two patients, the blood vascular systems were clinically normal. The vascular trees of 9 adult feet were injected through the dorsalis pedis artery with Berlin blue using the method described by Trueta and Harrison (1953). Similarly, an arm and a leg of a full-term foetus were injected through the subclavian and femoral arteries respectively. It was not found possible to obtain capillary filling in younger foetal specimens, only the arteries in the limbs being filled. Complete filling was obtained in adult and full term foetal limbs. In the preparation of the adult toes, the plantar skin was removed down to the distal bony phalanx and the remainder of the specimen was fixed in formalin. After fixation, partial decalcification with formic acid was carried out and then the distal phalanx was cut away. In order to investigate the capillary blood supply, thick sections (150–250 μ) were prepared from the formalin-fixed frozen tissue or from celloidin embedded tissue; these sections were then examined without additional procedures after clearing in xylol. Thin sections (8–25 μ) were prepared from celloidin or paraffin embedded material or from formalin-fixed frozen tissue. These were stained by various methods, including haematoxylin and eosin, periodic acid-Schiff, toluidine blue, Feulgen and silver proteinate. In staining for sulphydryl groups, the tissues were fixed in acidified alcohol as recommended by Barnett and Seligman (1952). In formalin-fixed tissue also, this method revealed great detail of the nail plate keratinization. The Spalteholz technique was used to demonstrate the complete arterial supply in digits from foetal specimens.

RESULTS.

Both haematoxylin and eosin and the silver proteinate staining gave useful information on the formation of the nail. Thin sections of foetal digits stained with haematoxylin and eosin demonstrated that the nail formed from the traditional matrix. In adult specimens there was no good evidence that the roof of the nail fold contributed more than a very small amount to the nail plate. No evidence was found of true nail formation from the nail bed distal to the lunula. In some adult toes, under pathological conditions, material was added to the nail plate from almost the whole inner surface of the nail fold and also from the nail bed below the visible part of the nail (Fig. 1). This material, comparable to the dorsal and ventral nails of Lewis, superficially resembled nail plate keratin. However the arrangement of the cells and the staining characteristics clearly showed it to be different. Silver proteinate staining gave a green-blue coloration, which faded into gold, at the level of the matrix. Only the gold colour is said to be characteristic of the intermediate nail of Lewis derived from the traditional nail matrix. The roof and floor of the nail fold gave rise only to keratinized material which stained blue green. These findings confirm and extend those of Lewis with regard to the tinctorial properties of the nail structure utilizing the silver proteinate method.

Stain for sulphydryl groups in adult toes showed the keratogenous zone of Giroud (Giroud and Bulliard, 1930) near the apex of the nail fold as a homogeneous circular area of deep blue staining cells (Fig. 2) whilst more distally it became a ring. Below the nail plate, a narrow blue band was seen indicating the presence

of many sulphhydryl groups. In foetal nails, the whole nail plate stained blue so that a distinct keratogenous zone could not be defined. This method of staining

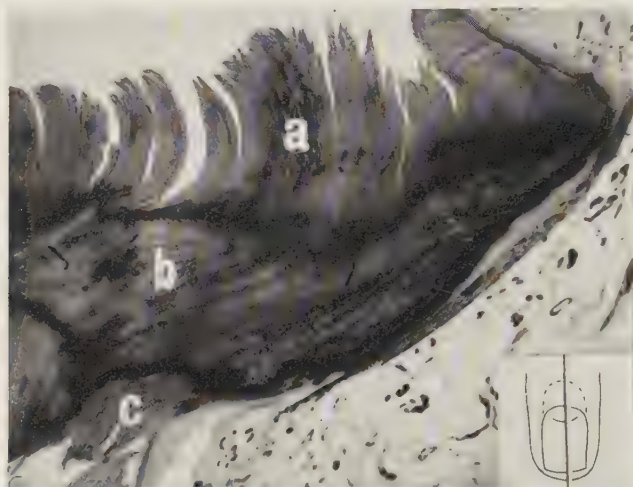


FIG. 1.—Hypertrophied nail stained to show sulphhydryl groups. Clearly distinguishes the three portions of the nail: dorsal (*a*), intermediate (*b*) and ventral (*c*).



FIG. 2.—Cross section through proximal area of matrix stained to show sulphhydryl groups. Dark area is the keratogenous zone.

best demonstrated the difference in structure of the true nail from the portions derived from the roof of the nail fold and nail bed (Fig. 1). In grossly hypertrophied nails the individual cells of the true nail showed blue outlines and they were flattened parallel to the long axis of the nail. On the other hand,

the cell outlines of the dorsal and ventral components did not stain blue but could be seen as much less flattened individual cells. Likewise, staining with the periodic acid-Schiff technique outlined the individual cells and confirmed the different arrangements described above. In several specimens, material rich in sulphydryl groups could be seen at the lateral edge of the nail plate derived from the lateral nail folds.

By injection of the vascular tree it was seen that the arterial supply to the nail matrix originates from the two main arterial arches lying below the nail plate. The arteries forming these arches are branches of the digital arteries after they reach the pulp space of the terminal phalanx. Each passes dorsally from the palmar aspect around the thin waist of the distal phalanx: in this region it is in a confined space bounded medially by the bone and laterally by a dense ligament which extends from the ungual process anteriorly to the lateral ligament of the distal interphalangeal joint behind (Flint, 1955). On emerging from this space, the artery divides, one branch anastomosing with its fellow of the opposite side to form a distal arcade and the other to form the proximal arcade. The proximal arcade also receives a contribution from the middle segment of the finger as a vessel passing over the distal interphalangeal joint. This was described by Flint (1955) and was clearly seen in foetal digits.

The capillary blood supply to the tissues around the nail is abundant. Similar to the capillary loop system of the skin, there exists a capillary loop system supplying the whole of the nail fold (Figs. 3 and 4). A small area beneath the lunula was found where the loop system was ill-defined but this may have been due to poor filling in the specimens examined.

In two grossly hypertrophied nails, the epidermis of the nail bed was seen to be broken through and granulation tissue rich in a capillaries protruded into the nail plate. On several occasions, epidermal cysts were found beneath the nail plate: these were of various sizes, the largest being about 1 mm. in diameter (Fig. 5). They arose from the rete forming the nail bed. Keratin formed and collected centrally within the cysts. In one specimen, keratinized material was added to the nail plate proper in a region overlying the cysts although these were distal to the site of the traditional matrix. The nail plate contained pigment which was displaced by the keratinized material added from the nail bed and the sides of the nail fold. The cause of these cysts is unknown. It is likely that constant trauma from tight footwear may be responsible.

CONCLUSIONS.

The findings of Lewis (1953 and 1954) occasionally have been confirmed. It appears certain that nail may form from three sites but this is probably pathological.

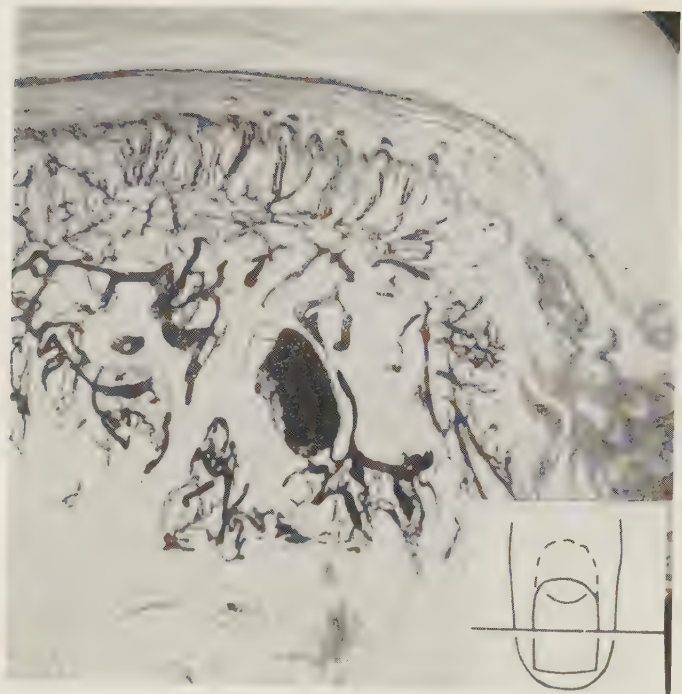


FIG. 3. Capillary loops below nail plate.

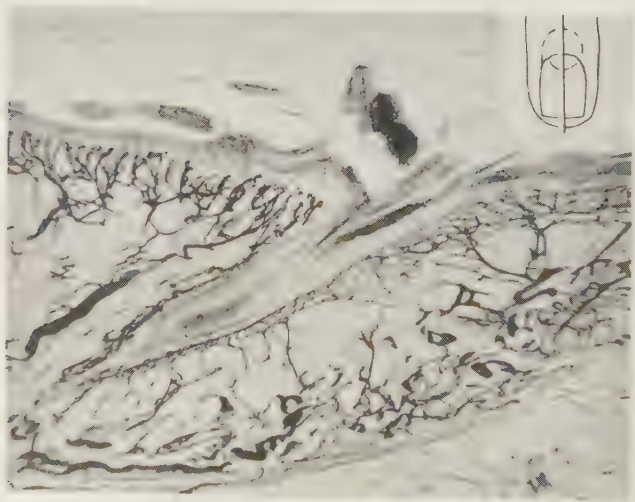


FIG. 4.—Capillary loop system of nail fold. Thick section.

In adult toes, keratinized material closely resembling true nail may be added to the nail plate from the roof of the nail fold and from the nail bed distal to the matrix. This is not true nail as is shown by its different staining properties, especially with silver proteinate and stains for sulphhydryl groups, and by the different morphology of its individual cells. However, it appears very similar to true nail and the ability of these sites to produce such material must be considered when gross pathological changes occur in the nail plate. These additional sites of nail keratin production are probably stimulated into activity by inflammation or trauma. Similarly, keratin cysts arising from the nail bed are probably due to the latter.



FIG. 5.—Multiple cysts below nail plate. Haematoxylin; blood vessels injected.

The arterial blood supply of the nail is mainly derived from the digital arteries *via* the pulp space of the terminal phalanx. It has been confirmed that there is an additional supply from the middle segment of the digit passing over the dorsum of the terminal interphalangeal joint.

The capillary blood supply of the nail fold including the matrix consists of the loop system seen in normal skin, but the loops are shorter. Below the distal part of the nail plate, however, the loops resemble those of the skin proper. In the vicinity of the lunula the capillary supply is less clearly defined than elsewhere. The presence of capillary loops throughout the nail fold would support the theory that nail growth may take place at all sites within it.

My thanks are due to Dr. W. E. Ehrlich, Pathologist in Chief of the Philadelphia General Hospital, for the supply of adult limbs, to Miss R. Bloomberg for technical assistance, to Mr. E. F. Glifort for the illustrations and to Dr. George Hambrick jr. for much help and advice.

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PAPER CHROMATOGRAPHY OF HUMAN HAIR FOLLICLES AND HAIR EXTRACTS.

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HISTOCHEMICAL methods have been widely used in studies on the hair follicle but the possibilities of paper chromatography to detect extractable substances have been less fully exploited. Ellis, Gillespie and Lindley (1950) made use of the method in their work on wool follicles which were obtained in considerable quantity by Ellis' (1948) wax technique for removing hair roots from excised skin. In the present work, much smaller amounts of material obtained by plucking human scalp hair are shown to be accessible to analysis by paper chromatography. It was hoped that by this procedure chemical differences associated with pigmentary variations might be detected.

EXAMINATION OF HAIR FOLLICLES.

The bulb of each active follicle was cut off with a razor blade and squashed on the starting point of the chromatographic paper (Whatman No. 1) immediately after plucking. Fifty follicles were used routinely in each preparation although amino-acids can be detected with as few as ten. Follicles from a number of subjects were examined but the most intensive and varied tests were done on those from a man with medium blond hair, who is referred to in the text as the main subject.

Amino-acid pattern.—Two-dimensional runs on $7\frac{1}{2}$ in. squares proved adequate for separating amino-acids. The papers were folded into cylinders and ascending runs in closed glass tanks were performed, using water-saturated phenol as the first solvent and lutidine-water (lutidine 15 parts, water 6.75 parts) as the second. The colours were developed with ninhydrin. The identification of amino-acids was made on the basis of published maps, by comparison with runs of known compounds and by characteristic colour reactions in some instances. Follicles from five males with the following hair colours, black (1), dark brown (2), medium blond (1) and strong red (1) and three females, medium brown (2) and blonde (1) were examined by this procedure.

The pattern of ninhydrin-positive spots was essentially similar in all subjects (Fig. 1). The strongest spots were those of taurine (*Ta*) and glutamic acid (*Glu*). Aspartic acid (*As*) and glycine (*Gly*), with characteristic ninhydrin colours, and alanine (*Al*) and glutamine (*Gm*) were also prominent. Two fainter spots with relatively high R_F values in both solvents were identified as leucine (*Leu*) and valine (*Val*) by comparison with these compounds in long one-dimensional runs using as solvents butanol, acetic acid and water (4 : 1 : 5). Neither phenylalanine nor methionine was detected in these experiments. A trace of serine (*Se*) was sometimes found and a constant small greyish spot below glutamic acid was thought to be ethanolamine phosphate (*Ep*). A faint unidentified spot was sometimes found below glycine. Tests for tyrosine with diazotised sulphanilic acid on butanol-acetic acid (BuA) runs were negative. A faint reaction for tryptophane with the Ehrlich reagent (paradimethylaminobenzaldehyde) was obtained if BuA papers were run in the dark. No spots suggestive of cystine or related sulphur compounds were observed.

A rough estimate of the amount of glutamic acid in the follicle was obtained by weighing fifty follicles after drying (0.35 mg.) and by running known amounts of this compound in parallel for comparison with follicle chromatograms. The result suggests that the amount of glutamic acid is in the region of 5–10 $\mu\text{g.}$ representing about 1.5–3.0% of the dry weight of the follicle.

Tests for particular compounds.—The suggestion has been made (Fitzpatrick *et al.*, 1958) that red hair pigments, like the ommochromes of some invertebrates, are derived from tryptophane. The detection of kynurenin, 3-hydroxy-kynurenin or other indole derivatives in the hair follicles would therefore be of interest. Follicles from four subjects, two with strongly red hair and two with darker shades of red,

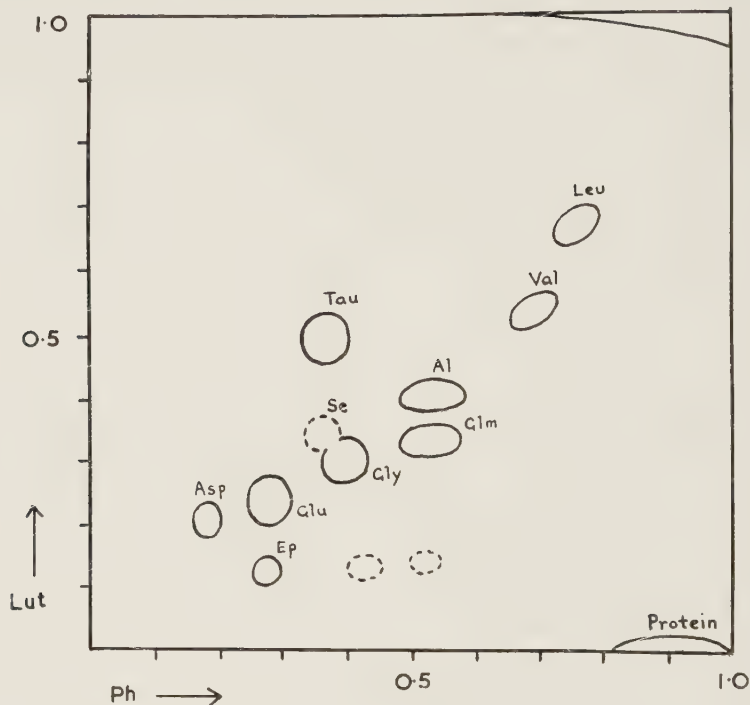


FIG. 1.—Pattern of ninhydrin positive spots from human hair follicles. First solvent phenol, second solvent lutidine.

were run in BuA with tryptophane, kynurenin, 3-hydroxy-kynurenin and 3,4 dihydroxyphenylalanine (dopa) on the same paper. Neither kynurenin nor 3-hydroxy-kynurenin was detected in the hair follicle runs by fluorescence in ultraviolet light although less than 1.0 $\mu\text{g.}$ of kynurenin is detectable in this way. The Ehrlich reaction was also negative for these and other compounds except tryptophane. Follicles from two additional red-haired subjects were run on the same papers as follicles from the main subject and from an Indian, together with the above-mentioned reference substances. Ultraviolet fluorescence was again negative in all the follicle runs. The papers were treated with the p-nitraniline reagent (Smith, 1958) which gives a strong reaction with dopa and with 3-hydroxy-kynurenin. Neither of these substances was detected in the hair follicles, though in each case faint reddish streaks were noted between the dopa level and the starting point. Dopa gives a blue ninhydrin spot in a well-isolated position in phenol/lutidine chromatograms, but no trace of it was

observed in any of the runs by this method. Indications of reducing substances with R_F values close to and lower than that of dopa (0.2) were noted in runs on the main subject in BuA when developed with ammoniacal silver nitrate (Smith, 1958) or with the Folin-Ciocalteu reagent. The main silver-reducing spot from the follicles ran close to glucose when run on the same paper in BuA, propanol-ammonia (Smith, 1958) or lutidine-water. Further identification was not attempted.

MELANOMA TISSUE.

It was thought that evidence of pigment intermediates might perhaps be obtained from highly pigmented Harding-Passey melanoma tissue. Pieces of the tumour were stored in the deep freeze ($-20^\circ\text{C}.$) and fragments were squashed on the chromatographic paper as required. Phenol-lutidine runs developed with ninhydrin showed a pattern of amino-acid spots similar to human follicles, except that the presence of aspartic acid and glutamine was more doubtful. Neither tyrosine nor dopa was detected in BuA runs treated with the Pauly reagent, but a trace of tryptophane was found after treatment with the Ehrlich reagent. There was no indication of kynurenin or its derivatives.

EXAMINATION OF HAIR EXTRACTS.

Bolliger (1951) has described the extraction of small amounts of various substances from animal and human hair, and Rebell (1957) has shown that kynurenin occurs in aqueous extracts of rat hair. It seemed possible, therefore, the compounds involved in pigment formation might remain in trace amounts in the mature shaft. There was also the possibility that individual chemical variations, which might have a genetic basis, would be revealed.

The hair was washed in warm soapy water, thoroughly rinsed in distilled water and dried. 120 mg. portions were placed in 7.0 ml. of distilled water and heated for 2 hours at $100^\circ\text{C}.$ on a water bath. The extract was evaporated to dryness under vacuum at $60-65^\circ\text{C}.$ and the residue was dissolved either in a few drops of water or, more usually, in butanol acidified with hydrochloric acid. The solutions were pale yellow. Two-dimensional ascending chromatograms were run in water-saturated phenol and butanol-pyridine (Smith, 1958). A wide range of hair colours was examined in this way; the details are given below.

Hair colour.	Males.	Females.
Red	11	3
Dark brown	3	1
Medium blonde	3	2
Blonde	1	0
Albino	0	2
Black (Indian)	4	1
.. (W. African)	1	0
	23	9

The position of ninhydrin positive spots based on average R_F values is shown in Fig. 2. Aspartic acid, glutamic acid, glycine, alanine, tyrosine and leucine were detected in all specimens. The presence of phenylalanine, which runs close to leucine, is uncertain. Long runs on a few specimens in BuA or in butanol-pyridine confirmed the presence of valine and absence of methionine, but although the presence of phenylalanine was suspected, it was not fully separated. Valine, however, was not detected in three of the thirty-two two-dimensional runs and threonine was not found in four; serine, which is usually a powerful spot, was doubtfully present in two

specimens. A spot (*Cit*) lying close to alanine, was tentatively identified as citrulline; it was often faint and was not found in two specimens. Extracts from two dark-haired subjects were run in BuA with citrulline as a reference. With the Ehrlich reagent citrulline gave a slow yellow colour which gradually changed to a purple-brown; fainter spots at the same level in the hair extract runs also reacted with a slow yellow changing later to a faint reddish brown.

One or more spots were sometimes found below aspartic acid in phenol/butanol-pyridine runs (Fig. 2: 1 and 2); these may represent cystine or cysteine. Other faint, unidentified spots were noted in a few subjects. A well-marked ninhydrin-

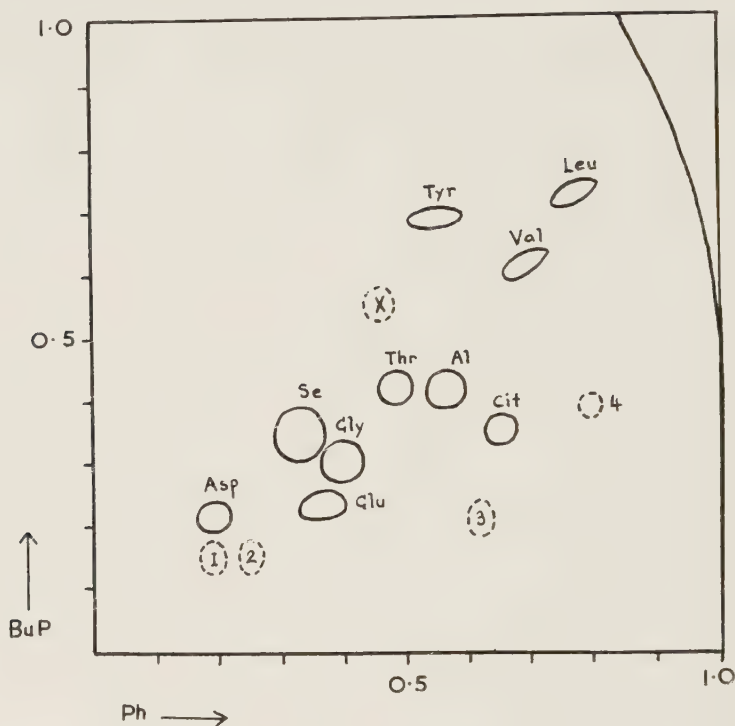


FIG. 2.—Pattern of ninhydrin positive spots from aqueous extracts of human hair. First solvent butanol-pyridine, second solvent phenol.

positive spot (Fig. 2: X) was noted in two subjects below tyrosine and confirmed in several subsequent runs. Since one subject was an Indian, extracts from four other Indians were run and a faint indication of this spot, the identity of which is unknown, was noted in one of them.

No definite indication was obtained that the variations in amino-acid pattern described above were correlated with the colour of the hair.

EXAMINATION FOR KYNURENIN AND RELATED COMPOUNDS.

In view of Rebell's findings, hair extracts from eight European albino subjects were run in BuA and the paper examined under ultra-violet light and after treatment with the Ehrlich reagent. No spots corresponding to kynurenin, 3-hydroxy-kynurenin or related substances were detected, though tryptophane was observed in two spe-

cimens. Some of the albino extracts and also ones from pigmented subjects—dark (2), medium blonde (2), blonde (2) and red (3)—were run in BuA followed by saturated aqueous potassium chloride (Dalglish, 1956). Again, neither kynurenin nor its derivatives was detected though the presence of the former in albino rat hair was readily confirmed. Tryptophane was detected in some specimens and not in others, but its incidence did not appear to be related to hair colour. All the specimens showed the presence of urea, giving a strong yellow spot with Ehrlich's reagent.

DISCUSSION.

It seems virtually certain that the amino-acids which can be extracted chromatographically from the hair follicle must be present in the free state. The possibility that some spots represent peptides has not, however, been eliminated. Both the composition of the amino-acid mixture and the concentration seem to rule out the likelihood that one is dealing with a simple tissue fluid transudate; indeed, amounts of human serum greatly exceeding the volume of the hair follicle specimens give only faint indications of a more limited range of amino-acids. Some of the amino-acids identified from hair follicles seem, in fact, to be widely distributed tissue constituents as may be seen from the data on the free amino-acids of various tissues summarised by Meister (1957).

The failure to demonstrate dopa, a well-established melanin precursor, in active pigment-forming follicles presumably means that this and later intermediates do not accumulate to a detectable extent but are rapidly removed as insoluble polymer. The negative results for tryptophane derivatives do not of course exclude the possibility that these are involved in the formation of certain hair pigments.

There are several notable differences in the amino-acid patterns obtained from hair follicles and from mature hair. The follicles yield taurine and glutamine as strong spots and small amounts of ethanolamine phosphate, but these are not found in hair extracts, while the latter always contain tyrosine and usually threonine and citrulline which were not detected in the follicles. The hair extracts also contain relatively more serine and perhaps small amounts of cystine derivatives.

The possibility that the amino-acids in hair extracts may be contaminants derived from sweat presents a difficulty. Dent and Walshe (1954) report that paper chromatograms of human sweat contain a considerable range of amino acids, including those found in hair extracts, and in particular citrulline as a strong spot. This spot, however, is faint in hair extracts and these do not show histidine, which is a strong spot in sweat. It is unlikely that contaminants on the hair surface are involved, since it was found that when hair was soaked in acidified butanol for twenty-four hours no amino-acids could be detected chromatographically in the solvent. This alcohol is said not to penetrate hair

readily but it would be expected to remove material on the surface. The occurrence of citrulline in hair extracts may not in any case indicate sweat contamination, since Rogers and Simmonds (1958) have shown that this substance is present in hydrolysates of the inner root sheath of wool follicles. Although contamination cannot be decisively eliminated as a possibility, it seems likely that much of the extractable material in the hair shaft is a residue incorporated in the shaft when the cells undergo the changes of keratinisation. If it can be assumed that the extraction procedure is efficient, it appears, judging by the weights of material taken and the intensity of the spots, that the amino-acids of the shaft are present in much lower concentrations than they are in the active follicles.

The apparent individual variations in hair extract patterns might repay more detailed study. Only small amounts of hair have been used in these experiments since quantities of this order are most readily obtainable, but extracts of larger amounts could be suitably fractionated for the detection of substances present in higher dilution and there is also scope for the study of other groups of compounds.

SUMMARY.

1. The patterns of freely extractable amino-acids in the active human hair follicle and in the mature hair shaft are described and compared.
2. Certain individual variations in the amino-acid patterns of hair extracts were noted; these did not appear to be correlated with differences in hair pigmentation.
3. Neither tyrosine nor tryptophane derivatives related to the synthesis of pigments were detected in the follicles or hair extracts.

The writer wishes to thank Professor C. E. Dent for valuable advice and Dr. C. E. Dalgliesh for a gift of kynurenin and 3-hydroxy-kynurenin.

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THE DIAMETER AND GROWTH PHASE OF HAIR IN RELATION TO AGE.

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AND

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A PILOT study was made of normal human scalp hair at various ages from birth to senescence in an attempt to correlate the size and growth phase of hair with age. In general, the results on size confirmed those of Trotter and Duggins (1948) as the diameter attained a maximum at about the age of ten. After the age of twenty, the percentage of "small hairs" tended to increase. Regardless of the patient's age, most hairs were in the active growth phase.

MATERIALS AND METHODS.

The study was carried out on histological sections of autopsy material. The twenty subjects ranged in age from prematurity to eighty-eight. One to four specimens were obtained from each decade. Examination of sections permitted measurement of the maximum diameter of the hair shafts and establishment of the growth phase of the follicles.

All specimens were obtained from the vertex of the scalp at the edge of the usual incision at autopsy and were fixed in Helley's solution. After fixation, pieces 5 mm. square were utilized for microscopic study. Sections were cut parallel to the skin surface at various levels and stained with toluidine blue. Hairs were then examined for maximum diameter and growth phase. In actively growing hairs, the internal root sheath is preserved from the bulb to the level of the sebaceous-duct orifice. Resting hair has no internal root sheath. Differentiation was made by study of sections at the lower level of the sebaceous gland. The diameter of the hair was measured by an ocular micrometer.

RESULTS.

The average size of hair at various ages is shown in Fig. 1. Hairs varied in diameter from 0.027 mm. to 0.09 mm. The average diameter during the first year of life was 0.04 mm. The average diameter in a child aged nine was 0.065 mm. Minor variations in average hair diameter (0.058 to 0.069 mm.) were noted in the older age groups.

The proportion of "small hairs" at various ages is shown in Fig. 2. Hairs with a diameter of 0.027 mm. or less were arbitrarily considered "small hairs." This term was used in preference to vellous or lanugo hair since the only distinction was size. Small hairs constituted 77% during the first year of life.

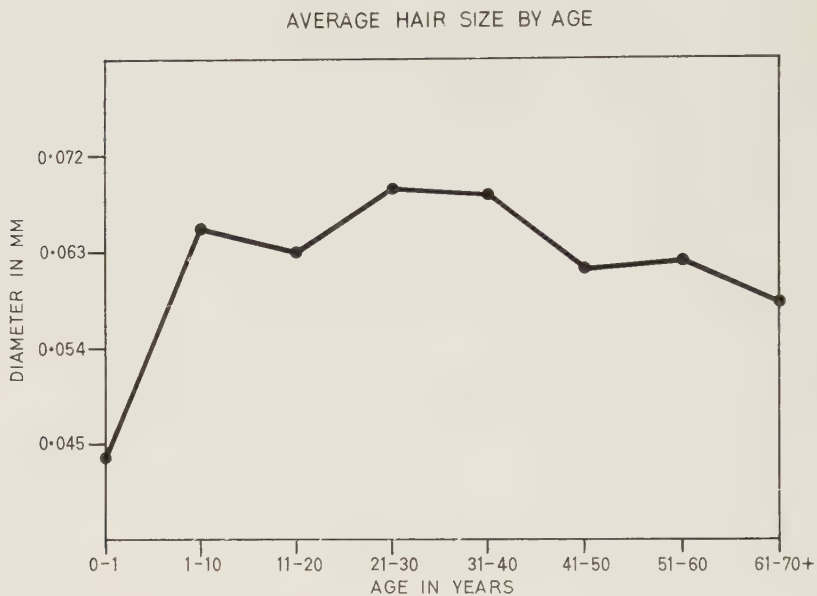


FIG. 1.

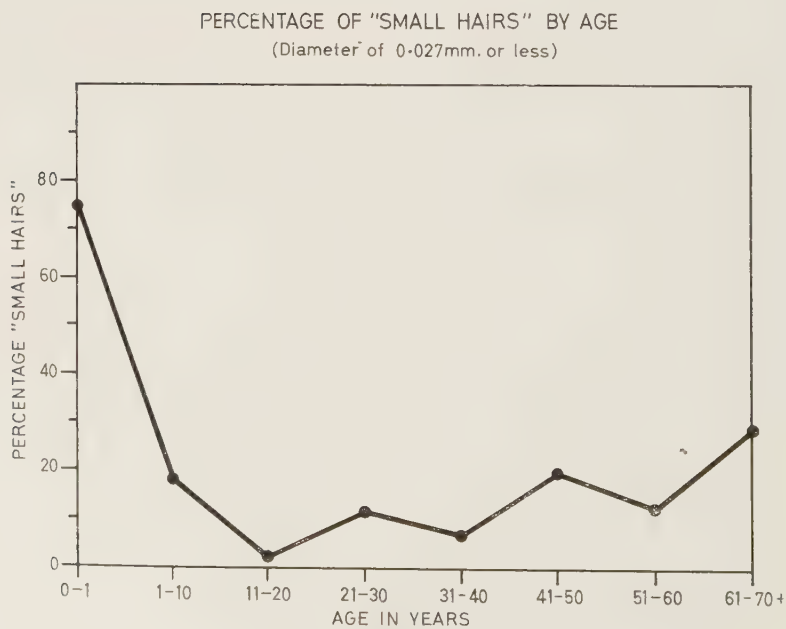


FIG. 2.

In subsequent decades, the proportion of small hairs varied between 2 and 28%. A tendency to higher percentages was noted in the older age groups.

The percentage of resting hairs by age is depicted in Fig. 3. In the first decade of life, the percentage of resting hairs varied between 6 and 11. In the next three decades, the percentage remained stabilized between 18 and 22. Curiously enough, the proportion of resting hairs was somewhat lower in the older age groups (forty-one years or more).

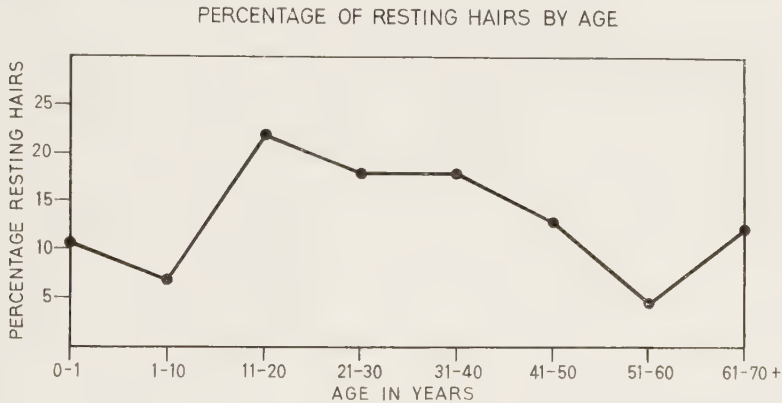


FIG. 3.

SUMMARY.

A study of the diameter and growth phase of hairs was made from scalp sections in twenty autopsies. The subjects varied in age from prematurity to 88 years.

Hair diameters varied from 0.02 mm. to 0.09 mm., with averages of 0.04 mm. in the first year of life and 0.065 mm. in subjects ten or more years of age.

In the first year of life, 77 per cent of hairs were small (0.027 mm. or less in diameter). The percentage of "small hairs" varied between 2 and 28 in subsequent decades, being somewhat higher in the older groups. The higher percentage of "small hairs" in older subjects is in agreement with the general trend toward structural atrophy with advancing age.

The percentage of resting scalp hairs varied between 4.5 and 22. It was low in the first decade, about 20 in the next three decades, and decreased in the older groups. The diminution in the percentage of resting hairs in older subjects indicates either that the growth phase is prolonged beyond the normal span or that telogen-type hairs are more rapidly shed.

This project was supported by a grant from Ayerst Laboratories, New York.

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ROYAL SOCIETY OF MEDICINE.

SECTION OF DERMATOLOGY.

President—Dr. JOHN T. INGRAM.

20 November 1958.

(1) **Necrobiosis Lipoidica.** (2) **Livedo Reticularis.**—Dr. E. LIPMAN COHEN.
V.B. female aged 11.

History.—For 10 months she has had on the left leg a lesion which does not itch.

Clinical findings.—In the left leg there is a firm, thick, shiny yellow plaque. On both thighs and slightly on the legs there is mild livedo.

Investigations.—Glucose tolerance test—20 June 1958: Blood sugar (Fasting) 134 mg.%. Urine sugar nil.; 40 g. glucose given. Blood sugar ($1\frac{1}{2}$ hours) 122 mg.%. Urine sugar nil. Blood sugar ($2\frac{1}{2}$ hours) 85 mg.%. Urine sugar nil.

Biopsy.—Necrobiosis lipoidica (Dr. H. Haber).

Comment.—I am showing this patient and the next because of the rarity of this disease in children. Examples of necrobiosis lipoidica in children have been described including one by Hildebrand and his colleagues (1940) in a girl age 7, but I have been unable to find an account of this condition in a non-diabetic child.

The occurrence of necrobiosis lipoidica and livedo in this child is probably coincidental. The association of the two conditions is mentioned in neither of the two recent reviews on livedo—Grinspan and Zurita (1957) and Farber and Barnes (1955).

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HILDEBRAND, A. G., MONTGOMERY, H. and RYNEARSON, E. H. (1940) *Arch. int. Med.*, **66**, 851.

Necrobiosis Lipoidica.—Dr. E. LIPMAN COHEN.

M.R.W. female aged 13.

History.—For $1\frac{1}{2}$ years she has had on the left shin a lesion which does not itch. It is growing slowly.

Clinical findings.—On the left shin there is a small, red, flat, palpable demarcated plaque with telangiectasis on the surface. The redness disappears on diascopy.

Investigations.—Urine: no sugar (tested on two occasions).

Biopsy: Necrobiosis lipoidica. (Dr. H. Haber).

DISCUSSION.

THE PRESIDENT: I do think that diabetes often supervenes in these cases of necrobiosis in children.

Dr. A. BARLOW: Is it not wise to put such cases on a low carbohydrate diet? May they not be in a prediabetic state?

Dr. BRIAN RUSSELL: I have seen diabetes develop in an adolescent some three years after the onset of his necrobiosis.

Dr. G. A. HODGSON: As regards treatment, Plaquenil might improve the condition. We had such a lesion in a diabetic which has responded extremely well to that.

Dr. P. J. FEENY: I have had a case of necrobiosis for twenty years. The woman is now aged 65; she is not diabetic, but she had a younger sister who was diabetic.

Dr. RODERICK HOWELL: I saw a patient at St. Mary's, Paddington, who had necrobiosis lipoidica with a normal glucose tolerance curve. Two years later, when she attended St. Thomas's she was found to have diabetes.

Dr. R. M. B. MACKENNA: Marten and Dulake injected hydrocortisone with good results. About three months ago I put an elderly non-diabetic woman on to 250 mg. of vitamin E daily for a month and the lesions were perceptibly softer; I followed with hydrocortisone injections. She is still under treatment but the lesions have been reduced in size and thickness.

THE PRESIDENT: I have tried vitamin E in two or three cases without effect.

Dr. E. LIPMAN COHEN: Thank you for the suggestion. My only comment would be about glucose tolerance tests on these children, I would need a lot of convincing that it would be of sufficient benefit to involve the child in a series of pricks. It is a bad thing to do unless there is good reason for so doing.

REFERENCE.

MARTEN, R. H., and DULAKE, M. (1957) *Brit. J. Derm.*, **69**, 395.

The following cases have been reported in *Proc. R. Soc. Med.* **52**, 367.

Erythema Gydatum Repens.—Dr. J. SCHNEEWEISS (for Dr. S. C. GOLD).

Alopecia Mucinoso (Pinkus).—Dr. C. J. STEVENSON.

The following cases were also shown :

Solitary Mast Cell Tumour.—Dr. E. LIPMAN COHEN.

Congenital Epidermolysis Bullosa.—Dr. B. BENTLEY PHILLIPS.

Pityriasis Lichenoides et Varioliformis.—Dr. J. G. HOLMES.

Granuloma Annulare of Feet, with features suggesting sarcoid.—Dr. Denis SHARVILL.

Pityriasis Rubra Pilaris Tylosis starting at Age 19.—Dr. P. J. Feeny and Dr. N. A. THORNE.

Lichen Myxodermatosus.—Dr. A. PORTER.

Angio-lupoid.—Dr. J. H. ALLISON (for Dr. F. RAY BETTLEY).

Hamartoma in a Boy of 10, Composed of Blood Vessel, Lymph Vessel, Fatty and Fibrous Tissue.—Dr. J. S. PEGUM.

Pachyonychia Congenita. A Case with an Associated Dystrophy of the Teeth.—Dr. E. WILSON JONES and Dr. H. J. WALLACE.

(1) **Chondrodermatitis Nodularis Helicis Chronica in a Housewife.**—Dr. BEATRICE LEWIS.

(2) **Prurigo Nodularis with Recovery.**—Dr. BEATRICE LEWIS.

Acute Lupus Erythematosus and Pulmonary Tuberculosis.—Dr. N. A. THORNE and Dr. A. GREENBERG (for Dr. R. H. S. HURST).

Mr. J. S. Calnan and Dr. B. Rossatti read a paper on "Some Observations on the Histopathology of Chondrodermatitis Nodularis Helicis Chronica".

18 December 1958.

Elastoma Intrapapillare Perforans (Miescher).—Dr. HAROLD WILSON.

W.T., male, aged 10.

History.—Four years ago red, scaling, circular lesions appeared on the left side of his neck with a few lesions on the ear. They were treated with fungicides without response. They had been slow in development and were completely asymptomatic.

Past history.—Osteomyelitis of right humerus when aged 18 months. Malaria on three or four occasions. Measles and chicken pox.

Family history.—Mother was suffering from malaria when she was pregnant. Grandmother diabetic. One sister alive and well. No consanguinity in the family.

Clinical findings.—On the left side of the back of the neck is a red, papular scaling eruption of circinate pattern with smaller similar lesions on the ear.

Investigations.—Microscopic examination of scales—no fungus found.

Histology.—Several papules showed characteristic condensation of elastic fibres. In follicles degenerated elastica and necrobiotic tissue are being pushed to the surface. The histology is typical of Miescher's elastoma.

I think that these patients are nearly all small children and they do finally get over the condition. There is some controversy as to whether this is primarily a degeneration of the elastic tissue or whether it is in fact primarily an affection of the sweat ducts or the hair follicles or even the lanugo hair.

Multiple Angiomata.—Dr. ETAIN CRONIN (for Dr. H. J. WALLACE).

E.S. female, aged 49 years. Cook.

History.—Four months ago this patient first noticed red spots in the right loin. They subsequently spread over the whole body, sparing the face, hands and feet. She thinks she is still developing new lesions.

Her general health has been good except for mild menopausal symptoms for which she took stilboestrol 1½ mg. daily for one month before the eruption appeared.

Clinical findings.—Over the trunk and limbs are numerous small red raised lesions varying in diameter from 1–3 mm. The largest ones have the typical appearance of Campbell de Morgan spots.

General examination.—Nothing abnormal was found.

Investigations.—Chest skiagram normal. Blood count normal. Liver function tests normal.

Biopsy.—"Haemangioma".

Comment.—The large number of lesions present and their eruption over a short space of time is unusual. The smaller ones bear a superficial resemblance to petechiae.

DISCUSSION.

Dr. H. J. WALLACE: This lady had had oestrogens some months previously. I have not seen similar eruptive angiomata previously.

Dr. J. MORGAN: I have seen a lady aged about 35 with a condition like this. She seemed to be in perfect health.

THE PRESIDENT: I recollect what I thought was a late developing naevus, a similar type of eruption, in a girl aged 15, and I have a woman aged 50, who suffers from a familial muscular dystrophy, with a similar eruption over her chest and breasts. I do not feel that this is the same as occurs in pregnancy or at the menopause or from liver damage.

Lichen Amyloidosis.—Dr. D. C. G. BETT (for Dr. LOUIS FORMAN).

P.P. (male) aged 43. Decorator.

History.—Ten years itchy eruption on trunk and limbs. Originally diagnosed as lichen planus in 1949. No previous skin trouble. General health always good. No history of drug-taking.

Family history.—No skin diseases known. Sister has asthma. Father alive and well. Mother died of tuberculosis when patient aged 4.

Examination.—Brownish lichenoid papules on trunk especially central back and chest and sacral region. Also scattered on limbs and neck. A good deal of brownish pigmentation also.

C.V.S.—Clinically normal. Blood pressure 135/90.

Respiratory system.—Clinically normal.

Abdomen.—N.A.D.

Urine.—No albumen or sugar.

Investigations.—Chest skiagram: Lung fields clear. Blood count: Haemoglobin 96%, W.B.C. 5,200. Normal differential count. E.S.R.: 2 mm. in 1 hour.

Biopsy: Lichen amyloidosis (Dr. H. Haber).

DISCUSSION.

THE PRESIDENT: Is the distribution on the chest and back uncommon?

Dr. F. B. DOWLING: That is the point, are not the lesions usually larger on the legs where they usually occur, these are only on the back and chest. That does not matter, but I cannot understand it not being diagnosed.

Dr. G. C. WELLS : There is a moulage of a similar case at the Institute of Dermatology, showing these lichenoid pigmented papules scattered over the chest. The legs were never affected. That eruption had persisted for twenty years, it was moderately itchy, and its histology was similar to that of the case shown to-day.

THE PRESIDENT : Is the pigmentation melanin?

Dr. H. HABER : Yes, melanin.

Lichenoid Reticulosis of the Nape.—Dr. W. N. GOLDSMITH.

A woman aged 31.

About seven years ago, when she was under a long emotional strain, itchy, pea-sized papules appeared on the back of her neck. The condition persisted and extended. One year later a diagnosis of lichen simplex was made and the patch was given three exposures of X-rays, each of 300 r. A few months later, it was described as a large patch of neurodermatitis on the right side of the neck and there had been no improve-

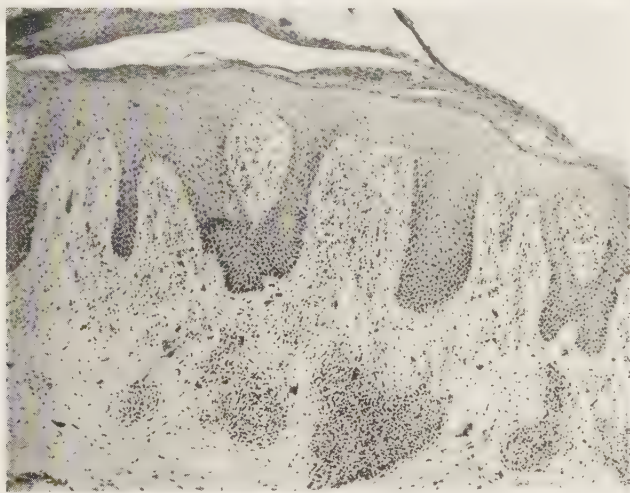


FIG. 1.

ment. She first consulted me in August 1958. In the interval, the trouble had got worse and more extensive and had been uninfluenced by marriage, residence in the West Indies and by numerous applications including tar and hydrocortisone. She stated that the eruption was itchy but that she did not rub it.

Examination.—The back of the neck, below the hair margin, was almost covered by two broad bands, closely similar to lichen simplex except that they were composed throughout of tiny, closely-set yet discrete papules. No glands or spleen felt.

Investigations.—W.R. negative. Blood-count : white cells 7300, neutros. 59·5% ; lymphos. 28% ; monos. 9% ; eos. 3·5%.

Biopsy : A fairly copious mixed cellular infiltrate throughout the dermis. There are some clumps of large cells with vesicular nuclei. The epidermis is in places somewhat thickened and parakeratotic (Figs. 1 and 2). No amyloid or mucin.

Treatment and Progress.—Rapid response to course of Grenz rays. Most of the eruption cleared and itching completely vanished after three doses of Grenz rays, each 14 kV, 500 r, at fortnightly intervals. A narrow strip down the right side required two further doses.

Comment.—The histopathology and the clinical picture seem at variance. Whether this is a neurodermatitis or a reticulosis, it is remarkable that it should have responded

so well to Grenz rays, having been entirely uninfluenced by quite heavy dosage of X-rays. It is particularly surprising in view of the depth of the infiltrate.

Dr. H. HABER: Montgomery described many years ago the presence of multinucleate cells in lichen simplex. They are probably derived from the endothelium of blood vessels and I always look for them in making a diagnosis of lichen simplex.



FIG. 2.

The following cases have been reported in *Proc. R. Soc. Med.* 52, 367.

Indeterminate Leprosy.—Dr. W. H. JOPLING.

Unusually Widespread Tinea Corporis.—Dr. C. M. RIDLEY.

The Following cases were also shown :

Infantile Eczema Treated by Psychotherapy.—Dr. H. JULIE ALTSCHULOVA (for Dr. P. J. HARE).

Lifelong Besnier's Prurigo Treated by Weekly Psychotherapy.—Dr. H. JULIE ALTSCHULOVA (for Dr. W. N. GOLDSMITH).

(1) **Hypodermite Nodulaire with Uveitis. ?Periarteritis Nodosa.**—Dr. P. J. HARE.

(2) **Hypodermite Nodulaire. ?Bazin's Disease in Man.**—Dr. P. J. HARE.

Scarring Nail Dystrophy.—Dr. E. WILSON JONES.

CURRENT LITERATURE.

HISTOCHEMISTRY OF PLANTAR HYPERKERATOSES. A CONTRIBUTION TO THE PHYSIOLOGY OF THE SKIN SURFACE. G. K. STEIGLEDER. (1958) *J. invest. Derm.*, **31**, 29.

Methods for detecting nonspecific esterase in various components of the cutaneous surface are described in detail. J. H. T. D.

HYDROLYZING ENZYMES IN MACROPHAGES. G. C. WELLS. (1958) *J. Invest. Derm.*, **31**, 83.

Esterases take part in the phagocytosis of *Leishmania*, which has no lipid envelope, just as they do in leprosy and tubercle. The coelomic phagocytes of *lumbricus* also take esterase with their indian ink. J. H. T. D.

THE PRESENCE OF VITAMIN D PRECURSORS IN HUMAN EPIDERMIS. V. R. WHEATLEY and R. P. REINERTSON. (1958) *J. invest. Derm.*, **31**, 51.

Since it was not considered practicable to remove the surface lipid before extracting that contained in the whole epidermis the amount was calculated on the basis of Hodgson Jones and Wheatley's sebum level measurements and estimated to comprise about a third of the total. The evidence here presented shows that the human epidermis contains enough pro-vitamin D for 3 sq. inches of skin to provide the daily requirement of 400 I.U. This removes the need to suppose that vitamin D has to be reabsorbed from the surface or ingested as in the case of birds and animals with thick dark pelts. J. H. T. D.

CUTANEOUS HYPERSENSITIVITY TO ADHESIVE AND SCOTCH TAPE. J. C. MURPHY, A. E. REIF and H. L. JANUARY. (1958) *J. invest. Derm.*, **31**, 45.

The analysis of an allergy resulting from a therapeutic accident (sustained by one of the authors who, having applied an adhesive bandage to his sprained ankle, renewed it daily "in the interest of personal hygiene" until restrained by the development of a generalized exudative dermatitis) points to diamyl hydroquinone contained both in the strapping applied to the ankle and in Scotch tape subsequently employed to secure an eye shield. It is recommended that a solvent be used when adhesive tape must be repeatedly changed, preferably propylene glycol ethyl ether. J. H. T. D.

CHEMICAL DATA ON HUMAN EPIDERMAL KERATINIZATION AND DIFFERENTIATION. P. FLESCHE. (1958) *J. invest. Derm.*, **31**, 63.

A well documented review of recent work on the genesis and composition of the horny layer emphasizing the distinction that must be made between keratinization and "differentiation" by which is designated the make-up of soluble and non-fibrous components. J. H. T. D.

THE FAILURE OF CHLOROQUINE TO STIMULATE THE HUMAN ADRENAL CORTEX. W. B. SHELLEY and R. P. ARTHUR. (1958) *J. invest. Derm.*, **31**, 109.

Chloroquine, still considered the most effective and least toxic antimalarial and also widely used in dermatology, accumulates in liver, spleen, kidney, lungs and haematocytes in concentrations 200-700 times that of the plasma level after prolonged administration. In guinea pigs the adrenal cortex becomes hypertrophied. Atrophy of the testis has been reported in rats.

Eosinophil counts were watched during a 28-day course of 250 mg. t.d.s. administered to 8 healthy males, 8 controls receiving a dummy. No difference. While the urinary 17-hydroxycorticoids remained within normal limits, the 17 ketosteroids were reduced in 5 of the subjects. J. H. T. D.

THE INTERFERENCE PHENOMENON IN ALLERGIC CONTACT DERMATITIS. W. L. EPSTEIN and A. M. KLIGMAN. (1958) *J. invest. Derm.*, **31**, 103.

The allergens in their ascertained order of potency, 2 : 4 dinitrochlorobenzene, paranitrosodimethyl aniline, the monobenzyl ether of hydroquinone and *krameria* were used in this experiment. It had been found possible over a number of years to work out the likelihood of producing sensitization to each of these materials at various concentrations, by the method of open patch

test—that is to say a standard quantity of acetone containing different amounts of the allergen is evaporated in a cup 2.9 cm. in diameter.

It is found that a more potent allergen will inhibit sensitization to a weaker one provided that the former is employed at a concentration yielding maximum rates of sensitization (not necessarily the highest possible concentration) and the latter in a considerably weaker concentration. But a less potent allergen will not in any circumstances block sensitization to a stronger one.

J. H. T. D.

EVIDENCE FOR A HUMORAL MECHANISM WHICH PREVENTS GROWTH OF DERMATOPHYTES. A. L. LORINCZ, J. O. PRIESTLEY and P. H. JACOBS. (1958) *J. invest. Derm.*, **31**, 15.

Dialysis bags of cellulose paper 1 centimetre in diameter were filled with a suspension of trichophyton mentagrophytes in distilled water. If placed on Sabouraud's medium or in human serum sufficient nutrient material would penetrate the 0.3 micron pores to allow the microbe to grow but if the serum is changed often enough or if the bag is put in a mouse's peritoneal cavity something manages to diffuse into it and stop the fungus growing.

J. H. T. D.

USE OF LINDANE TO CONTROL MITES IN FUNGUS CULTURES. C. NIBLEY and A. NEWTON. (1958) *J. invest. Derm.*, **28**, 373.

Dispersion of 0.01% of a wettable lindane powder in the culture medium does not interfere with the growth of pathogenic fungi other than *Blastomyces dermatitidis* and *histoplasma capsulatum*; but it is rapidly lethal to tyrophagus and other mites which in their search for nourishment make an untidy mess of the cultures.

To prevent infestation it is probably more convenient to intercept the mites, but for the salvage of already infested cultures this method should prove useful.

J. H. T. D.

AN EFFECT OF PANTOTHENIC ACID ON SERUM COPPER VALUES IN HUMAN PELLAGRA. G. H. FINDLAY and I. J. VENTER. (1958) *J. invest. Derm.*, **31**, 11.

That elevation of the serum copper, as estimated spectrophotometrically by means of bis-cyclohexanoneoxalyldihydrazone, may be an index of pantothenic acid deficiency in man is suggested by the effect of that accessory food factor in promptly reducing to normal levels the serum copper of Bantus suffering from pellagra. Nicotinamide neither has this effect by itself nor does it influence the effect of pantothenic acid.

J. H. T. D.

OXIDATIVE METABOLISM IN PERFUSED SURVIVING DOG SKIN. R. L. BELL, R. LUNDQUIST and K. M. Halprin. (1958) *J. invest. Derm.*, **31**, 13.

The skin covering the medial aspect of a dog's thigh, excised and perfused by way of the saphenous artery and vein, continues to oxidize carbon acetate supplied in a suitable perfusion fluid for 18 hours.

J. H. T. D.

STUDIES OF THE MECHANISM OF ALLERGIC ECZEMATOUS CONTACT DERMATITIS.

II. USE OF C¹⁴ LABELLED 2 : 4 DINITROCHLOROBENZENE IN GUINEA PIGS. V. H. WITTEN and C. H. MARCH. (1958) *J. invest. Derm.*, **31**, 97.

To the skin of sensitized animals radioactive 2 : 4 dinitrochlorobenzene was applied; but in order to match the intensity of the reaction the sites in the controls were prepared with tincture of cantharides.

In the sensitized animals the C¹⁴ disappeared from the skin and accumulated in the buffy coat of the heart blood in an appreciably shorter time.

J. H. T. D.

MYCOLOGICAL FLORA OF THE HEALTHY EXTERNAL AUDITORY CANAL: A STUDY OF 120 HUMAN SUBJECTS. W. A. LEA, D. S. SCHUSTER and E. R. HARRELL. (1958) *J. invest. Derm.*, **31**, 137.

An earhole of each of 87 males and 33 females was swabbed with a damp cotton-tipped applicator which was then smeared on a Sabouraud glucose agar slope. Identifiable fungi grown from 23 of the males and 4 of the females included *candida albicans* (10%) other varieties of *candida* (10%) *hormodendrum* (20%) and 10 others. It is concluded therefore that the presence of fungi does not necessarily imply their participation in inflammatory processes. (Although the sex difference is not regarded as significant it might have been worthwhile to compare the flora on left and right.)

J. H. T. D.

STRUCTURAL CHANGES IN BURIED HUMAN SKIN. W. L. EPSTEIN and A. M. KLIGMAN. (1958) *J. invest. Derm.*, **28**, 377.

The events are not at all clearly described. The recipient tissue reacted more or less violently with a polymorphonuclear infiltrate, replaced during 14 days by lymphocytes giant cells histiocytes new vessels and hyperplasia of the neighbouring covering epithelium. Quite substantial parts of the transplants survived; epithelial outgrowths showed an "urge to cover denuded surfaces"; hair follicles "reacquired their normal histologic structure and produced growing hairs" sebaceous glands after a phase of dedifferentiation resumed their normal appearance. But the photomicrographs illustrate only the expected disorganization of appendages and horn cyst formation. We are not told the size of the autotransplants and we are certainly not shown them flourishing *in situ*.

J. H. T. D.

LANGERHANS CELLS AND THE MODIFIED TECHNIC OF GOLD IMPREGNATION BY FERREIRA-MARQUES. J. FAN and ROSIE HUNTER. (1958) *J. invest. Derm.*, **31**, 115.

From a comparison of Langerhans cells melanocytes and the nerve endings in muscle impregnated with gold, the impression is gained that many of the appearances previously supposed to indicate specific structure may well be adventitious. The method and intensity of reduction influences the appearance of nonspecific deposits.

J. H. T. D.

THE EFFECTS OF P³² ON THE MAST CELLS IN THE SKIN OF RATS. J. FAN. (1958) *J. invest. Derm.*, **30**, 167.

The mast cells of the rat—ordinarily fairly constant in appearance—though they are not reduced in number undergo changes in shape and loss of metachromatic granules during the first 7 days after an intraperitoneal dose of P³² calculated to be lethal to 50% within 30 days.

J. H. T. D.

FOX-FORDYCE WITH HIDRADENITIS SUPPURATIVA. R. F. SPILLER and J. M. KNOX. (1958) *J. invest. Derm.*, **31**, 127.

[It is impossible to agree that the authors have established any important relationship between a rare functional disorder of the apocrine stoma and an extremely common complication of axillary itching.]

J. H. T. D.

RAPID SEROLOGICAL DIFFERENTIATION OF CANDIDA ALBICANS FROM CANDIDA STELLATOIDEA. M. A. GORDON. (1958) *J. invest. Derm.*, **31**, 123.

The serum globulin, of a rabbit which 10 days previously had received an intravenous injection of heat-killed candida, can be conjugated with fluorescein isocyanate and impart fluorescence to the microbe with which it agglutinatively reacts.

J. H. T. D.

THE PENETRATION OF AN ANTICHOLINESTERASE AGENT (SARIN) INTO SKIN. II. AUTORADIOGRAPHIC STUDIES. I. H. BLANK, R. D. GRIESEMER and EDITH GOULD. (1958) *J. invest. Derm.*, **30**, 187.

The evidence here produced shows that isopropyl methyl phosphorofluoride penetrates the covering epithelium with little facilitation by the hair follicles though greatly helped by superficial scratches.

J. H. T. D.

PENETRATION OF RADIOIODIDE (I¹³¹) THROUGH HUMAN SKIN. J. TAS and Y. FEIGE. (1958) *J. invest. Derm.*, **30**, 193.

About 2% of the iodine applied to a 16 sq. cm. area of skin on the flexor aspect of the forearm and 0.3% of that applied to the palm was excreted in the urine or could be detected by a Geiger counter over the thyroid.

J. H. T. D.

OBSERVATIONS ON SUBMERGED GROWTH AND DEAMINATION OF AMINO ACIDS BY DERMATOPHYTES. ADELE M. BURACK and S. G. KNIGHT. (1958) *J. invest. Derm.*, **30**, 197.

Several well-known dermatophytes grow luxuriantly in shake culture. The maximum production of ammonia from amino acid is at pH 7 and it ceases below pH 4.5.

J. H. T. D.

A STUDY OF THE ACTION OF CHYMOTRYPSIN ON THE SKIN. A. Scott. (1958) *J. invest. Derm.*, **30**, 201.

Epidermis may be separated from dermis *in vitro* by exposure for an hour to chymotrypsin 1 mg. per ml. of Ringer's solution. Pretreatment of the skin with hydrocortisone ointment inhibits the effect to an extent varying somewhat with the age of the subject from whom the skin was taken.

Chymotrypsin is supposed to attack ground substance: at first that between basal layer and rete, later at the dermo-epidermal junction and finally between the collagen fibres: in other words electing ground substance that is imperfectly polymerized. Steroid may possibly act by reducing permeability. J. H. T. D.

ADRENOCORTICAL DYSFUNCTION IN THE EARLY STAGE OF PEMPHIGUS VULGARIS.

H. A. COHEN, T. D. ULLMAN and A. DOSTROVSKY. (1958) *J. invest. Derm.*, **30**, 207.

Tests designed to detect deficiencies in the salt- and water-regulating function of the adrenal cortex gave results only a little more than suggestive of cortical dysfunction in early pemphigus. J. H. T. D.

AN OBSERVATION IN ACROSCLEROSIS USING THE GALVANIC SKIN RESPONSE. D. J. PERRY, E. T. WRIGHT, G. E. MOUNT and CAROL M. LELAND. (1958) *J. invest. Derm.*, **30**, 173.

A normal galvanic skin response to Banthine, pilocarpine and placebo was observed in two patients with acroscclerosis, histological examination having shown an advanced degree of atrophy of the sweat apparatus. J. H. T. D.

EVALUATION OF THE TOPICAL THERAPEUTIC EFFICACY OF HEXETIDINE (STERISIL) IN VARIOUS DERMATOSES. A. L. WELSH and M. EDE. (1958) *J. invest. Derm.*, **30**, 177.

5-amino-1,3-bis (β ethylhexyl)-5-methyl hexahydropyrimidine provided that it is not dispersed in a fat-solvent vehicle can be safely employed as a placebo in a variety of dermatoses.

J. H. T. D.

STUDIES ON SERUM LIPIDS, PROTEINS AND LIPOPROTEINS IN PSORIASIS. W. A. LEA, H. H. CORNISH and W. D. BLOCK. (1958) *J. invest. Derm.*, **30**, 181.

Any abnormality of the serum lipids or lipoproteins among these 18 psoriatics could more readily be associated with concurrent atherosclerosis than with the psoriasis. J. H. T. D.

EFFECT OF ALTITUDE ON BASAL PALMAR SWEATING. PAMELA C. B. MACKINNON, I. L. MACKINNON and E. S. WILLIAMS. (1959) *Brit. med. J.*, **i**, 199.

Daily measurements of basal palmar sweating in four people living for a period of 12 days at altitudes of 6000 to 13,000 ft. (1830-3960 m.) in the Swiss Alps, and, in the following year, for a period of 40 days in the Karakoram of West Pakistan were recorded and compared with corresponding data for London. Sweating was found to be significantly less in the Alps and in the Karakoram than in London in all subjects.

Various possible reasons are discussed. It is suggested that reduction in basal sweating in high altitudes may be the result of stress causing reactions on the part of the pituitary and suprarenal glands with consequent increase in the blood levels of adrenocorticotrophic and corticoid endocrines. The administration of corticotrophin lowers the sweat index.

S. T. A.

LYMPHOCYTIC INFILTRATION OF THE SKIN. L. PRANDINA. (1958) *Cronache dell'I.D.I.*, **13**, 58.

A discussion on Jessner's lymphocytic infiltration of the skin with a description of two cases and comments on its relationship with other conditions characterized by aggregations of lymphocytes. The author mentions the wide variety of mechanical, chemical, toxic and infective stimuli that may give rise to lymphocytic reactions—in particular insect bites. D. S. W.

PALMAR-PLANTAR HYPERKERATOSIS, MAL DE MELEDA (CASE REPORT). A. GERARDI. (1958) *Cronache dell'I.D.I.*, **13**, 41.

The interest of this illustrated case report is in showing how different this condition is from the types of tylosis commonly seen in this country. The author discusses the possible relationship

with ainhum in regard to the isolated disseminate plaques and bands of hyperkeratosis that may occur.

D. S. W.

ACRODERMATITIS CHRONICA ATROPHICANS (HERXHEIMER). R. CAVALIERI. (1958) *Cronache dell'I.D.I.*, 13, 30.

Two cases are presented. Both had had severe exposure to cold before the onset of the disease. The author considers that the trauma of frost-bite may act in preparing the ground for the invasion of some unknown infectious organism responsible for the disease, and points to the greater incidence in northern latitudes.

D. S. W.

PILICA POLONICA. F. BAZZI. (1958) *Cronache dell'I.D.I.*, 13, 82.

An interesting short article on the epidemiology of this condition, which was first recorded among the Tartars of the thirteenth century. A violent epidemic in Poland in the fifteenth century established the name by which we know it and which is comparable to the "English Sweat" and, of course, the "French Pox" or "Neapolitan Disease". The tendency to fix another country's name to unpleasant diseases is a recognized form of gamesmanship, and the nature of plica polonica is no exception. The extreme crusting of the scalp and matting of the hair has generally been regarded as eczematous, but arising in the first place from lousiness. The epidemic nature of the outbreaks is striking and the author thinks that a louse-borne virus may have been the cause.

The article is well documented with footnotes, to which collectors of historical curiosities may refer.

D. S. W.

A DIFFUSE PAPULAR ERUPTION WITH LUPUS ERYTHEMATOSUS. R. CAVALIERI. (1957) *Cronache dell'I.D.I.*, 12, 146.

The author comments on cases shown by Gold and Gracianski and presents a third case, a man of 39. Since 1945 the patient had suffered from a severe light sensitivity of erythematous type, and following exposure to extreme sun in 1951 he developed a persistent fixed papular eruption on the arms, back and shoulders that lasted six years. There was an excellent response to chloroquin. Marked changes in the collagen were found histologically in the mid-dermis. The author discusses the relationship between the three cases.

D. S. W.

LUPUS ERYTHEMATOSUS TREATED WITH A COMBINATION OF QUINACRINE, HYDROXYCHLOROQUINE AND CHLOROQUINE. M. J. TYE, H. WHITE, B. APPEL, G. H. B. ANSELL. (1959) *New Engl. J. Med.*, 260, 63.

Forty-eight patients with chronic discoid lupus erythematosus and three with subacute systemic lupus erythematosus were treated with tablets containing quinacrine 25 mg., hydroxychloroquine 50 mg. and chloroquine 65 mg. The dose varied from 2-8 tablets daily, generally being 2.

In nineteen patients which chronic discoid lupus erythematosus the lesions disappeared, and in one with subacute systemic disease. Improvement occurred in a further twenty-five.

Relapse was noted in 13 of 20 patients in whom there had been complete clearing of lesions.

It is concluded that the simultaneous administration of 2 or 3 antimalarial drugs is more effective in some patients at certain times than any one of them alone.

A. D. P.

ALLERGIC REACTION TO POLIOMYELITIS VACCINE PROBABLY DUE TO PENICILLIN. L. PELNER. (1959) *New Engl. J. Med.*, 260, 230.

A patient developed pyrexia, urticaria, lymphadenopathy, exfoliative dermatitis and jaundice, after a dose of poliomyelitis vaccine. The reaction was attributed to penicillin in the vaccine.

A. D. P.

EFFECTS OF LINOLENIC AND STEARIC ACIDS ON CHOLESTEROL-INDUCED LIPOID DEPOSITION IN HUMAN AORTIC CELLS IN TISSUE-CULTURE. DAVID D. RUTSTEIN, E. F. INGENITO, J. M. CRAIG and M. MARTINELLI. (1958) *Lancet*, i, 545.

When cholesterol is added to the medium in which human aortic cells are being cultured, intracellular deposition of the lipid occurs in proportion to the amount of cholesterol added. Similar deposition occurs when cholesterol bound to β -lipoprotein is added to the culture medium. The intracellular lipid disappears in five days if the cholesterol containing medium is replaced

by normal medium. Simultaneous addition of linolenic acid with cholesterol completely inhibits the deposition of lipid, while the addition of stearic acid potentiates lipid deposition.

R. H. M.

PILONIDAL SINUS OF THE UMBILICUS. H. SADEGHI-NEJAD and A. J. H. RAINS. (1958) *Lancet*, i, 567.

A patient with a pilonidal sinus of the umbilicus is described and the various theories of aetiology are briefly mentioned.

R. H. M.

ALLERGIC SKIN RESPONSES ABOLISHED UNDER TREATMENT OF ASTHMA AND HAY-FEVER BY HYPNOSIS. A. A. MASON and S. BLACK. (1958) *Lancet*, i, 877.

During treatment for asthma and hay fever by hypnosis the urticarial responses of a patient's skin to scratch tests with pollen extracts showed progressive diminution, so that after seven weeks' treatment no response was obtained in the regular test site, i.e. the left arm. Tests in other parts of the skin were still strongly positive. After direct suggestion that the skin would be unresponsive anywhere on the body they were negative in all areas tested. A passive transfer test was carried out in a normal volunteer with positive results, so that antibodies were presumably still present in the patient's serum.

R. H. M.

TRIPLENNAMINE (PYRIBENZAMINE) IDIOSYNCRASY. H. E. BELLINGER. (1958) *Lancet*, i, 885.

A patient admitted to hospital in a coma had been treated for chronic rhinitis with tripelennamine. Before admission she had been ill for a few days with diarrhoea, headache and anorexia. After regaining consciousness she developed widespread urticaria which was controlled with antistamin and promethazine. She took tripelennamine again after leaving hospital and within two hours she had become disorientated and complained of nausea and vomiting. The author comments that this was a case of idiosyncrasy, distinct from toxic effects due to overdosage, although the effects produced were similar to those described in cases of overdosage.

R. H. M.

HAEMORRHAGIC DIATHESIS DUE TO INCREASED CAPILLARY FRAGILITY SECONDARY TO OVARIAN DEFICIENCY. R. WELLS. (1958) *Lancet*, i, 886.

A patient developed a haemorrhagic diathesis due to increased capillary fragility after oophorectomy. Increased capillary fragility has been found to occur just before menstruation and purpura may occur at these times. A non-thrombocytopenic haemorrhagic tendency has been described under the title of "hereditary familial purpura simplex", most cases occurring after the menopause. The patient described here responded well to oestrogen therapy, and the author suggests that hereditary familial purpura simplex may also be found to respond to oestrogens.

R. H. M.

HYPERSENSITIVITY TO HISTONE INDUCED EXPERIMENTALLY IN RABBITS. W. A. BARDAWIL, B. L. TOY and N. GALINS. (1958) *Lancet*, i, 888.

The abnormal γ -globulin found in patients with systemic lupus erythematosus, dermatomyositis and certain forms of rheumatoid arthritis has been shown to possess a specific reactive affinity for intranuclear desoxyribonucleoproteins in autologous, homologous and heterologous tissues.

This preliminary communication reports an attempt to sensitize rabbits to heterologous nucleoproteins in order to establish the immunological state supposed to exist in human patients. The antigen, consisting of commercial calf thymus histone together with an adjuvant, was injected into albino rabbits, other rabbits being injected with histone only and adjuvant only. One rabbit which received histone and adjuvant (the others died) showed antibodies to histone. This was demonstrated by the indirect fluorescent antibody "layering" technique. The serum antibody was shown by this technique to be bound histochemically with intranuclear material in autologous, homologous and heterologous tissues.

The authors suggest that the histochemical behaviour of this antibody is identical with that of the abnormal globulin, or so-called "L.E. factor" in human disease.

R. H. M.

PINK DISEASE AFTER APPLICATION OF MERCURY OINTMENT. M. E. R. STONEMAN. (1958) *Lancet*, i, 938.

Pink disease occurred in two siblings who had been treated for about two months with ung. hyd. ammon. B.P., applied to the perianal region only. Urine examination showed that they were

excreting considerable quantities of mercury but extensive inquiries did not reveal any source for this other than the ointment.

R. H. M.

GANGRENE OF THE EXTREMITIES FOLLOWING CARDIAC INFARCTION AND NORADRENALINE THERAPY. D. GREENBAUM. (1958) *Lancet*, i, 1103.

Symmetrical peripheral gangrene is a rare complication of heart disease with circulatory failure. It has been considered to be due to peripheral vasoconstriction as a reflex mechanism produced by a reduction in the stroke-volume of the heart. The patient described suffered from an extensive cardiac infarct and was treated with intravenous infusions of noradrenaline.

The author considers that the use of noradrenaline must have made an important contribution to the vasoconstriction that led to gangrene.

R. H. M.

THE SPREAD OF FATS AND OILY SUBSTANCES ON THE SKIN SURFACE. O. JACOBI. (1958) *Berufsdermatosen*, 6, 241.

The author investigated the spread of 20 different fats and oils on four different skin areas by means of a skin microscope and an analysis quartz lamp.

The tests were carried out on normal subjects. The spread is least marked on the forehead where sebum is most plentiful, most marked on the forearm which is poor in sebum secretion and intermediate values were obtained on the chest and upper arms. There were considerable individual variations which can also be explained by individual differences in sebum contents of the skin surface.

Because of these individual differences it is impossible to predict the spread of a fatty substance by determination of its surface tension only.

The spread of these substances on the skin surface is probably of great importance in the production of industrial dermatoses.

W. G. T.

INVESTIGATION OF THE BUFFER CAPACITY AND REACTION OF THE SKIN AFTER USE OF MODERN CLEANSING AGENTS. B. WERDELMANN. (1958) *Berufsdermatosen*, 6, 250.

Alkali and acid neutralization are measured and pH values determined after use of synthetic detergents on the forearms of normal test persons. Alkali neutralization is quicker than with toilet soap. Acid neutralization is uninfluenced by detergents. There is rapid restoration of the normal pH after use of detergents. In addition there were a number of incidental findings. There is alkalization of the skin as the result of insufficient rinsing. Hard water results in the production of potassium and magnesium soaps and removal of alkalinity. In the case of soft water alkalinity persists and has to be neutralized by physiological activity of the skin.

The alkalinity of soap solutions depends on the molecular weight. It increases with higher molecular weights. It was therefore assumed that the higher fatty acid soaps should produce greater alkali neutralization. This was not found to be the case, the neutralization time being in fact lengthened by increasing length of chains.

W. G. T.

RUBBER AND ECZEMA. (Conclusion of second communication.) E. SCHULTHEISS. (1958) *Berufsdermatosen*, 6, 267.

This is a comprehensive account of rubber technology likely to be of great value to the industrial medical officer and the dermatologist.

W. G. T.

UDDER SALVE DERMATITIS. J. A. v. PREYSS. (1958) *Berufsdermatosen*, 6, 287.

Fats and emulsions are finding increasing use in milking. In addition to reducing friction they are expected to have a germicidal action. For this purpose benzoates of aliphatic amines are often added and cases of dermatitis due to these substances are reported from time to time either in cowmen or in others who have access to the udder salves and use them for therapeutic purposes. Whereas the author of another paper on the subject considers the manifestation to be a toxic one the present author did not find any positive reactors in a control series and believes he is dealing with an allergic manifestation.

W. G. T.

SECOND DEGREE BURN DUE TO A LIQUEFIED HARDENER. A. RISSE-SUNDERMANN. (1958) *Berufsdermatosen*, 6, 290.

Epoxide resins are liquid substances which are mixed with liquid hardeners and the result is a solid, non-melting material. In addition to this cold method there is a thermoplastic method in

which the hardeners are polyamine fatty acid esters. These are highly viscous at room temperature. A workman had the dorsum of his hand accidentally covered with such hardener which set and could not be removed. After moistening with a mixture of ether and ethyl chloride in a proportion of 5 to 1 it was possible to remove the hardener and a second degree burn was found beneath which healed uneventfully.

W. G. T.

THE QUESTION OF LATE DAMAGE AFTER THE USE OF PNEUMATIC DRILLS. G. BREHM and H. HOLZMANN. (1958) *Berufsdermatosen*, 6, 309.

Vascular disturbances of the Raynaud's type have repeatedly been reported in those who have used pneumatic drills over long periods. A case is reported in which such changes occurred 7 years or more after the use of the pneumatic drill by the patient. Later the patient presented signs of early generalized scleroderma. It is considered possible that this condition was caused at least in part by the patient's previous employment.

W. G. T.

ANIMAL AND HUMAN SKIN TUBERCULOSIS. F. KRÖBER. (1958) *Berufsdermatosen*, 6, 317.

A full account of skin tuberculosis occurring in various domestic animals and the mode of transfer of this infection to human beings. Occasionally infection of animals by sufferers from human tuberculosis has also been reported.

W. G. T.

SOAPS CONTAINING ANTIMICROBIC AGENTS. H. P. FEILDER. (1958) *Berufsdermatosen*, 6, 328.

The term medicated soaps should be reserved for soaps containing agents of therapeutic value. Antimicrobial soaps are considered to have a cosmetic value only since they deodorise products of decomposition on the skin. A detailed list of antimicrobial agents added to soaps since 1945 is given.

W. G. T.

OCCUPATIONAL DERMATOSES—AN INTRODUCTION. COUNCIL ON INDUSTRIAL HEALTH. (1958) *J. Amer. med. Ass.*, 167, 1636.

Occupational diseases of the skin form the major proportion of all compensated industrial disease. At least 80% result from contact with primary irritant chemicals. Education and preventive measures can reduce the incidence. The variety of clinical lesions produced demands reasonable skill in dermatological diagnosis and a knowledge of potential hazards.

A. J. R.

OCCUPATIONAL DERMATOSES—PREDISPOSING AND DIRECT CAUSES. COUNCIL ON INDUSTRIAL HEALTH. (1958) *J. Amer. med. Ass.*, 167, 2203.

The anatomical and physiological characteristics of the skin on which the defence against noxious agents depend are sometimes tangible and obvious, sometimes intangible and obscure. Attention to the known factors will allow some predisposed persons to be excluded from certain occupations. Lack of cleanliness of the factory or the individual worker is a more important predisposing factor and one that can be reduced or eliminated. The direct causes of dermatitis are very briefly considered.

A. J. R.

THE PROBLEM OF PROLONGED AND RECURRENT INDUSTRIAL DERMATITIS. COUNCIL ON INDUSTRIAL HEALTH. (1958) *J. Amer. med. Ass.*, 168, 516.

Recurrence or persistence of occupational dermatitis is usually attributable to one or more of the following: incorrect diagnosis, failure to recognize the cause, failure to eliminate the cause, improper treatment, unrecognized secondary infection, lack of personal cleanliness, placement of dermatitis-prone individuals in hazardous jobs, development of cross-sensitivity, malingering.

A. J. R.

SOME MEDICOLEGAL ASPECTS OF OCCUPATIONAL DERMATOSES. COUNCIL ON INDUSTRIAL HEALTH. (1958) *J. Amer. med. Ass.*, 168, 1351.

This long paper has limited value for those practising beyond the frontiers of the United States, but those particularly concerned with compensation problems will find thought-provoking stimulation in the differences and similarities of British and United States law.

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